JC07 PCT/PTO 26 FEB 2002

FORM PTO-1390 (Modified) (REV 11-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	
753-10 PCT/US	
U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR	
10/070217	

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP99/06369	30 August 1999	30 August 1999

TITLE OF INVENTION

SYNTHESIS OF TEMPLATE-FIXED ß-HAIRPIN LOOP MIMETICS

APPLICANT(S) FOR DO/EO/US

John A. Robinson and Daniel Obrecht

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (24) indicated below.
4. ☐ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☒ is attached hereto (required only if not communicated by the International Bureau).
   b. ☐ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☐ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☒ A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:

13. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☒ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☒ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
20. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
21. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
22. ☒ Certificate of Mailing by Express Mail
23. ☒ Other items or information:

Copy of Submission of Duplicate Sequence Listing Diskette transmittal

U.S. APPLICATION NO (IF KNOWN, SEE 37 CFR)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
10/070217	PCT/EP99/06369	753-10 PCT/US

24. The following fees are submitted:.	CALCULATIONS PTO USE ONLY
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :	
☐ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$1040.00**	
☒ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$890.00**	
☐ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$740.00**	
☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$710.00**	
☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) **$100.00**	
ENTER APPROPRIATE BASIC FEE AMOUNT =	**$890.00**
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).	**$0.00**

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	9 - 20 =	0	x $18.00	**$0.00**	
Independent claims	1 - 3 =	0	x $84.00	**$0.00**	
Multiple Dependent Claims **(check if applicable)**			☐	**$0.00**	
TOTAL OF ABOVE CALCULATIONS			=	**$890.00**	
☐ Applicant claims small entity status. See 37 CFR 1.27). The fees indicated above are reduced by 1/2.				**$0.00**	
SUBTOTAL			=	**$890.00**	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)).			+	**$0.00**	
TOTAL NATIONAL FEE			=	**$890.00**	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).** ☒				**$40.00**	
TOTAL FEES ENCLOSED			=	**$930.00**	
				Amount to be: refunded	$
				charged	$

a. ☒ A check in the amount of **$930.00** to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **08-2461** A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Daniel A. Scola, Jr., Esq.
Hoffmann & Baron, LLP
6900 Jericho Turnpike
Syosset, New York 11791

SIGNATURE

Daniel A. Scola, Jr.
NAME

29,855
REGISTRATION NUMBER

2/26/02
DATE

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	ROBINSON, J.A. et al.	Examiner:	Unassigned
Serial No.:	To be assigned	Group Art Unit:	Unassigned
Filed:	Herewith	Docket:	753-10PCT/US
For:	Synthesis of Template-Fixed β-Hairpin Loop Mimetics	Dated:	February 26, 2002

I hereby certify this correspondence is being Deposited with the United States Postal Service as "Express Mail Post Office to Addressee" Mailing Label No EU203325616, addressed to Commissioner of Patents, Washington, D C 20231-0001

Dated February 26, 2002

Signature Linda J Schenle

Commissioner for Patents
Washington, DC 20231-0001

PRELIMINARY AMENDMENT PURSUANT TO 37 C.F.R §1.121(a)

Sir:

Please amend the above-identified application as follows:

IN THE SPECIFICATION:

Immediately after the title, please insert the following:

CROSS-REFERENCE TO RELATED APPLICATIONS

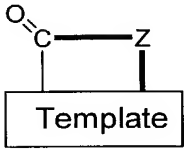
This application is the National Stage filing of PCT/EP99/06369 under 35 U.S.C. §371.

IN THE CLAIMS

Please delete claims 1-8 without prejudice and substitute therefore, as follows:

CLAIMS

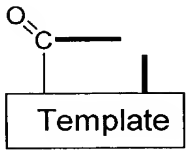
9. A process for the manufacture of compounds of the general formula



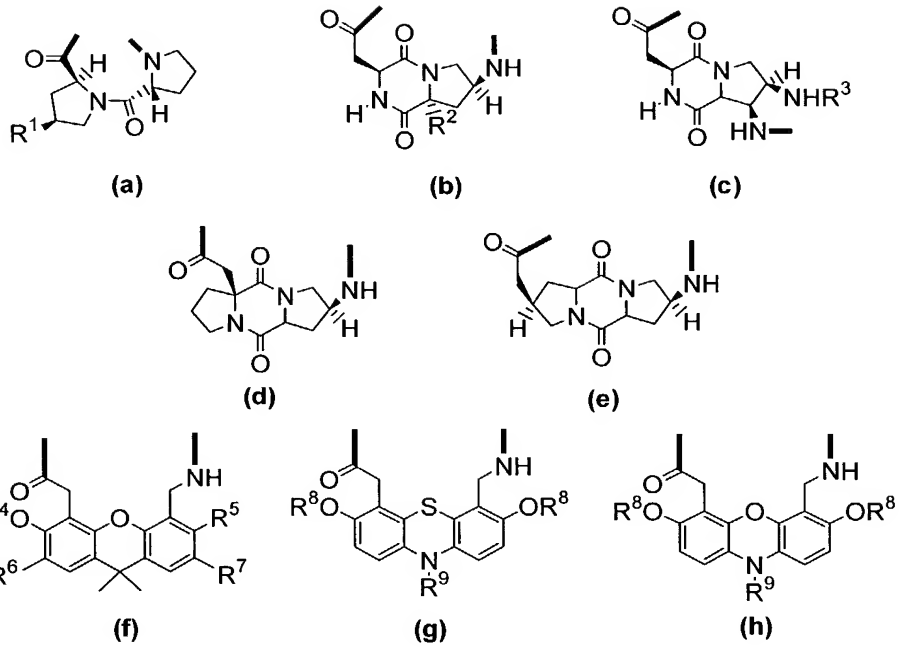
I

wherein

Z is a chain of n α-amino acid residues which, if their α-C atom is asymmetric, have L-configuration, n being an integer from 4 to 20, the positions of said amino acid residues in said chain being counted starting from the N-terminal amino acid;



is one of the groups of formulae



R^1 is hydrogen or a protected amino group;

R^2 is hydrogen or a group of formula CH_2-$COOR^{10}$;

R^3 is an amino-protecting group;

R^4 is lower alkyl or aryl-lower alkyl;

R^5 is lower alkyl, lower alkoxy or aryl;

R^6 is hydrogen, lower alkyl, substituted lower alkyl, aryl, Br or NO_2;

R^7 is hydrogen, lower alkyl, substituted lower alkyl, aryl, Br or NO_2;

R^8 is lower alkyl; substituted lower alkyl or aryl-lower alkyl;

R^9 is lower alkyl, substituted lower alkyl or aryl-lower alkyl; and

R^{10} is hydrogen, lower alkyl, substituted lower alkyl, aryl, aryl-lower alkyl, aroyl-lower alkyl or allyl;

and of salts thereof, which process is capable of being carried out as parallel array synthesis to yield libraries of numerous compounds of formula I in high yields and defined purities and which comprises

(a) coupling a solid support derived from polystyrene crosslinked with divinylbenzene which is functionalized by means of a 2-chlorotrityl linker with an appropriately N-protected derivative of that amino acid which in the desired end-product is in position $^n/_2$, $^n/_2+1$ or $^n/_2-1$ if n is an even number and, respectively, in position $^n/_2+^1/_2$ or $^n/_2-^1/_2$ if n is an odd number, any functional group which may be present in said N-protected amino acid derivative being likewise appropriately protected;

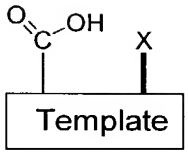
(b) removing the N-protecting group from the product thus obtained;

(c) coupling the product thus obtained with an appropriately N-protected derivative of that amino acid which in the desired end-product is one position nearer the N-terminal amino acid residue, any functional group which may be present in said N-protected amino acid derivative being likewise appropriately protected;

(d) removing the N-protecting group from the product thus obtained;

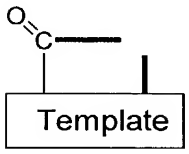
(e) repeating, if necessary, steps (c) and (d) until the N-terminal amino acid residue has been introduced;

(f) coupling the product thus obtained with a compound of the general formula

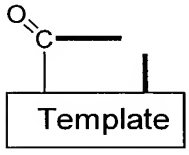


II

wherein

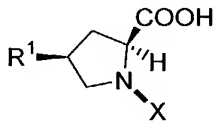


is as defined above and X is an N-protecting group or, if



is to be group (a), above, alternatively

(fa) coupling the product obtained in step (d) or (e) with a compound of the general formula **III**



III

wherein R^1 and X are as defined above;

(fb) removing the N-protecting group from the product thus obtained; and

(fc) coupling the product thus obtained with an appropriately N-protected

derivative of D-proline;

(g) removing the N-protecting group from the product obtained in step (f) or (fc);

(h) coupling the product thus obtained with an appropriately N-protected derivative of that amino acid which in the desired end-product is in position n, any functional group which may be present in said N-protected amino acid derivative being likewise appropriately protected;

(i) removing the N-protecting group from the product thus obtained;

(j) coupling the product thus obtained with an appropriately N-protected derivative of that amino acid which in the desired end-product is one position farther away from position n, any functional group which may be present in said N-protected amino acid derivative being likewise appropriately protected;

(k) removing the N-protecting group from the product thus obtained;

(l) repeating, if necessary, steps (j) and (k) until all amino acid residues have been introduced;

(m) detaching the product thus obtained from the solid support;

(n) cyclising the product cleaved from the solid support by means of O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate ("HATU") / 7-aza-1-hydroxybenzotriazole ("HOAt");

(o) removing any protecting groups present on functional groups of any members of the chain of amino acid residues and, if desired, any protecting group(s) which may in addition be present in the molecule; and

(p) optionally, converting the product thus obtained into a salt or converting a salt thus obtained into the corresponding free compound of formula I or into a different salt.

10. A process according to claim 9 wherein X and the N-protecting group of the amino acid derivatives is 9-fluorenylmethoxycarbonyl (Fmoc).

11. A modification of the process according to claim 9 for the manufacture of enantiomers of the compounds of formula I as defined in claim 9 in which all amino acids which have an asymmetric α-carbon atom are used in their D-Form and the enantiomer of a template corresponding to structure (a), (b), (c), (d) or (e) or a template corresponding to formula (f), (g) or (h) is used in step (f) and, respectively, the enantiomer of a compound of formula III is used in step (fa) and a derivative of L-proline is used in step (fc).

12. A process according to claim 9 which is carried out as parallel array synthesis to yield a library of numerous compounds of formula I as defined in claim 1 or enantiomers thereof.

13. A process according to claim 12 wherein the library comprises 24 to 192 compounds.

14. A process according to claim 13 wherein the library comprises 96 compounds.

15. A library of numerous compounds of the general formula I as defined in claim 9 or enantiomers thereof, obtainable by the process according to claim 12.

16. A library according to claim 15 comprising 24 to 192 compounds, obtainable by the process according to claim 13.

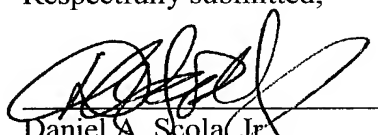
17. A library according to claim 16 comprising 96 compounds, obtainable by the process according to claim 14.

REMARKS

Claims 9-17 have been added. Claims 1-8 have been deleted to remove multiple dependencies. No new matter is believed to have been introduced with these claim amendments. The claim amendments are not narrowing amendments in response to 35 U.S.C. §§102, 103 or 112 concerns.

Applicant submits that no fees are required to make this amendment. However, if any fees are required, please charge same to Deposit Account No. 08-2461. Any questions regarding this matter may be directed to Applicants' undersigned counsel at the telephone number given below.

Respectfully submitted,



Daniel A. Scola, Jr.
Registration No.: 29,855
Attorney for Applicant(s)

HOFFMANN & BARON
6900 Jericho Turnpike
Syosset, New York 11791
(973) 331-1700

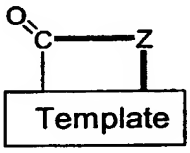
VERSION OF AMENDMENTS WITH MARKINGS SHOWING CHANGES MADE

IN THE CLAIMS

Claims 1-8 have been deleted. Claims 9-17 have been added.

Synthesis of Template-Fixed β-Hairpin Loop Mimetics

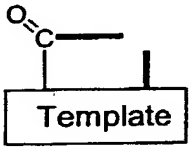
The present invention relates to a reliable process for the synthesis of template-fixed β-hairpin loop mimetics of the general formula



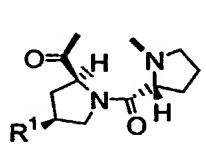
I

wherein

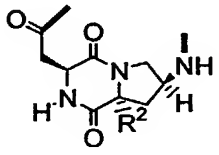
Z is a chain of n α-amino acid residues which, if their α-C atom is asymmetric, have L-configuration, n being an integer from 4 to 20, the positions of said amino acid residues in said chain being counted starting from the N-terminal amino acid;



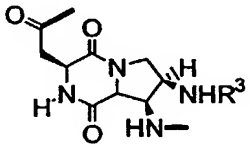
is one of the groups of formulae



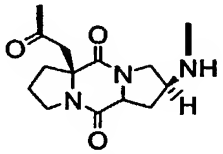
(a)



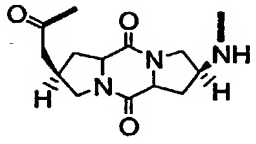
(b)



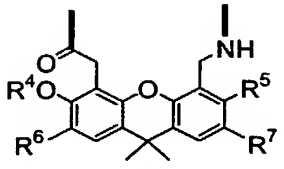
(c)



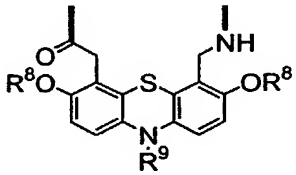
(d)



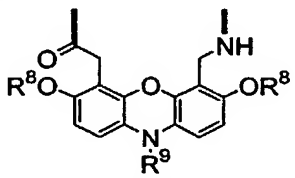
(e)



(f)



(g)



(h)

R^1 is hydrogen or a protected amino group;

R^2 is hydrogen or a group of formula CH_2-$COOR^{10}$;

R^3 is an amino-protecting group;

R^4 is lower alkyl or aryl-lower alkyl;

R^5 is lower alkyl, lower alkoxy or aryl;

R^6 is hydrogen, lower alkyl, substituted lower alkyl, aryl, Br or NO_2;

R^7 is hydrogen, lower alkyl, substituted lower alkyl, aryl, Br or NO_2;

R^8 is lower alkyl, substituted lower alkyl or aryl-lower alkyl;

R^9 is lower alkyl, substituted lower alkyl or aryl-lower alkyl; and

R^{10} is hydrogen, lower alkyl, substituted lower alkyl, aryl, aryl-lower alkyl, aroyl-lower alkyl or allyl;

and of salts thereof.

This process is based on a mixed solid- and solution phase synthetic strategy and comprises

(a) coupling an appropriately functionalized solid support with an appropriately N-protected derivative of that amino acid which in the desired end-product is in position $^n/_2$, $^n/_2$+1 or $^n/_2$-1 if n is an even number and, respectively, in position $^n/_2$+$^1/_2$ or $^n/_2$-$^1/_2$ if n is an odd number, any functional group which may be present in said N-protected amino acid derivative being likewise appropriately protected;

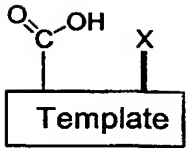
(b) removing the N-protecting group from the product thus obtained;

(c) coupling the product thus obtained with an appropriately N-protected derivative of that amino acid which in the desired end-product is one position nearer the N-terminal amino acid residue, any functional group which may be present in said N-protected amino acid derivative being likewise appropriately protected;

(d) removing the N-protecting group from the product thus obtained;

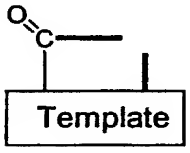
(e) repeating, if necessary, steps (c) and (d) until the N-terminal amino acid residue has been introduced;

(f) coupling the product thus obtained with a compound of the general formula

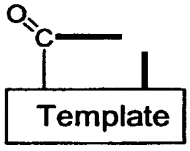


II

wherein

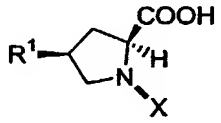


is as defined above and X is an N-protecting group or, if



is to be group (a), above, alternatively

(fa) coupling the product obtained in step (d) or (e) with a compound of the general formula **III**



III

wherein R^1 and X are as defined above;

(fb) removing the N-protecting group from the product thus obtained; and

(fc) coupling the product thus obtained with an appropriately N-protected derivative of D-proline;

(g) removing the N-protecting group from the product obtained in step (f) or (fc);

(h) coupling the product thus obtained with an appropriately N-protected derivative of that amino acid which in the desired end-product is in position n, any functional group which may be present in said N-protected amino acid derivative being likewise appropriately protected;

(i) removing the N-protecting group from the product thus obtained;

(j) coupling the product thus obtained with an appropriately N-protected derivative of that amino acid which in the desired end-product is one position farther away from position n, any functional group which may be present in said N-protected amino acid derivative being likewise appropriately protected;

(k) removing the N-protecting group from the product thus obtained;

(l) repeating, if necessary, steps (j) and (k) until all amino acid residues have been introduced;

(m) detaching the product thus obtained from the solid support;

(n) cyclising the product cleaved from the solid support;

(o) removing any protecting groups present on functional groups of any members of the chain of amino acid residues and, if desired, any protecting group(s) which may in addition be present in the molecule; and

(p) if desired, converting the product thus obtained into a salt or converting a salt thus obtained into the corresponding free compound of formula I or into a different salt.

The process of the invention can advantageously be carried out as parallel array synthesis to yield libraries of template-fixed β-hairpin loop mimetics of the above general formula I. Such parallel synthesis allows one to obtain arrays of numerous (normally 24 to 192, typically 96) cyclic template-fixed peptides of general formula I in high yields and defined purities, minimizing the formation of dimeric and polymeric by-products. The proper choice of the functionalized solid-support (i.e. solid support plus linker molecule), templates and site of cyclization play thereby key roles.

The β-hairpin loop mimetics of formula I can mimick flat surfaces of proteins and thus be used to probe large surface protein-protein interactions. They can serve as lead finding tools for protein targets where it is notoriously difficult to find small-molecular-weight lead compounds. Due to the structurally and conformationally well-defined architecture of the β-hairpin loop mimetics of general formula I, key amino acid residues or motifs can be integrated in conformationally locked arrangements. By shifting these key amino acid residues or motifs along the β-hairpin structure various conformations can be scanned (conformational scanning of key sequences). Alternatively, protein sequences can be mapped in order to detect β-hairpin loop motifs.

This technique in summary allows to determine rapidly key amino acids and motifs (hotspots) important for binding in large surface and flat protein interfaces not only in their sequential but also in their spatial arrangement. This information can ultimately be used for the design of small peptidomimetic drug candidates (Cunningham, B. C.; Wells, J. A. *Curr. Opin. Struct. Biol.* **1997**,

7, 457; Obrecht, D.; Altorfer, M.; Robinson, J. A. *Adv. Med. Chem.* Vol.4, 1-68, JAI Press Inc., 1999).

Due to the enormous advances in genomic sciences increasing numbers of biologically relevant proteins (e.g. receptors, enzymes, transcription factors, ligands, modulators, chaperones) are becoming available in pure form for structural and functional studies. This burst of novel biological targets has also created a need for sources of new organic molecules for pharmaceutical and agrochemical screening and also for more efficient screening technologies. Combinatorial and parallel chemistry have emerged in recent years to satisfy the increasing demand for new families of novel compounds (Obrecht, D.; Villalgordo, J.-M, "Solid- Supported Combinatorial and Parallel Synthesis of Small-Molecular-Weight Compound Libraries", *Tetrahedron Organic Chemistry Series*, Vol. 17, Pergamon, Elsevier Science, 1998).

While general screening of small-molecular-weight compounds (MG < 550) has successfully generated lead compounds for targets such as enzymes and receptors with well-defined binding sites and clefts, this technology gives rather poor results when ligand binding involves large surface protein-protein interactions with the corresponding receptors. These targets, however, are of increasing biological and pharmaceutical importance and many X-ray structures of such ligands, receptors and even ligands bound to their corresponding receptors are available. These include e.g. members of the growth factor family such as platelet-derived growth factor (PDGF) [Oefner, C; D'Arci, A.; Winkler, F. K.; Eggimann, B.; Hosang, M. *EMBO J.* 1992, *11*, 3921], nerve growth factor (NGF) [Ibanez, C. F.; Ebendahl, T.; Barbany, G.; Murray-Rust, J.; Blundell, T.; Perrson, H. *Cell*, 1992, *69*, 320-341], epidermal growth factor (EGF) [*Biochemistry* 1992, *31*, 236], basic fibroblast growth factor (b-FGF) [*Biochemistry* 1996, *35*, 2086], transforming growth factor βII (TGF βII) [Schlunegger & Grütter, *J. Mol. Biol.* 1993, *231*, 445], vascular endothelial growth factor (VEGF) [Müller et al., *Proc. Natl. Acad. Sci.* 1997, *94*, 7192], and members of the cytokine family such as the interleukines, tumor necrosis factor (TNFα and β) [Banner, D. W.; D'Arci, A.; Janes, W.; Gentz, R.; Schönfeld, H. J.; Broger, C.; Lötscher. H.; Lesslauer, W. *Cell*, 1993, *73*, 431-445]. Moreover, chemokines [Tarby, C. M.; Saunders, J. *Drug Discovery Today* 1999, *4*, 80-92; Ponath, P. D. *Exp. Opin. Invest. Drugs* 1998, *7*, 1-16] including members of the CC-family such as RANTES, MCP-1-4, Eotaxin and others, and the CXC-family such as GROα-γ, interleukine 8 (Il 8) and others have emerged as key mediators in a number of

inflammatory pathologies. In addition, integrines [see Obrecht, D.; Altorfer, M.; Robinson, J. A. *Adv. Med. Chem.* Vol.*4*, 1-68, JAI Press Inc., **1999**] play key roles in cell adhesion, migration and proliferation. All these protein ligands bind to their corresponding receptors involving one or several large surface interactions. Moreover, X-ray crystallography and site directed mutagenesis studies highlight the importance of surface β-hairpin loop motifs to be key in those interactions.

The anatomy of large surface protein interfaces has recently been analysed and the average contact surface was determined to be typically 600-900 A^2. The free energy of binding is not evenly distributed across the interfaces; instead, there are hot spots of binding energy made up of a small subset of residues in the dimer interface. These hot spots are enriched in tryptophan (Trp), tyrosine (Tyr) and arginine (Arg), and are surrounded by energetically less important residues that are most likely serving to occlude solvent from the hot spot [Bogan, A. A.; Thorn, K. S. *J. Mol. Biol.* **1998**, *280*, 1-9]. Occlusion of solvent is believed to be a necessary condition for highly energetic interactions. The β-hairpin loop motif offering two opposite β-sheet surfaces (e.g. a hydrophobic and a hydrophilic face) for possible binding interactions is ideally suited to meet these criteria for surface interactions.

The β-hairpin motif is very abundant in nature and occurs on the surface of many protein ligands and in the hypervariable domains of antibodies. The β-hairpin motif consists of two antiparallel β-strands linked by a short loop or turn and have been classified depending on the H-bonding network [Sibanda, B. L.; Blundell, T. L.; Thornton, J. M. *J. Mol. Biol.* **1989**, *206*, 759-777]. One example, par excellence, is found in the antigen binding sites of antibodies [Padlan, E. A. Mol. Immunol. 1994, 31, 169-217], which are composed of amino acid residues located in six so-called hypervariable loops or complementarity-determining-regions (CDR's), three each from the heavy- and light- chain variable regions (v_H and v_L). Of the six CDR loops in antibodies of the Ig family, four may be classified as β-hairpins connecting adjacent antiparallel β-sheets, two from the v_L domain, L_2 and L_3, and two from the v_H domain, H_2 and H_3. Recent estimates suggest that a large majority of L_1, L_2, L_3, H_1 and H_2 hypervariable regions maybe classified into one of 18 different canonical conformations [Chothia, C.; Lesk, A.; Gherardi, E.; Tomlinson, I. M.; Walter, G.; Marks, J. G.; Llewelyn, M. B.; Winter, G. *J. Mol. Biol.* **1992**, *227*, 799-817; Martin, A. C.; Thornton, J. M. J. Mol. Biol. **1996**, *263*, 800-815; Al-Lazikani, B.; Lesk, A.; Chothia, C. J. Mol. Biol. **1997**, *273*, 927-948].

The present invention provides a reliable process for the synthesis of template-fixed cyclic peptides of general formula I which mimick the various naturally occurring β-hairpin conformations, especially those present in growth factors, cytokines and chemokines, integrines and antibodies (see e.g. Figure, Example 1). Template structures corresponding to above formulae (a) through (h) have been shown to stabilize the H-bond network present in β-hairpins [e.g. for (a): Spaeth et al. *Helv. Chim. Acta* **1998**, *81*, 1726; Favre, M.; Moehle, K.; Jiang, L.; Pfeiffer, B.; Robinson, J. A. *J. Am. Chem. Soc.* **1999**, *121*, 2679-2685; for (b): Emery et al., *J. Chem. Soc. Chem. Comm.* **1996**, 2155; Bisang et al. *J. Am. Chem. Soc.* **1998**, *120*, 7439; for (c): Pfeifer, M. *J. Chem. Soc. Chem. Commun.* **1998**, 1977; for (d): Pfeifer et al. *Helv. Chim. Acta* **1997**, *80*, 1513; for (e): Beeli et al. *Helv. Chim. Acta* **1996**, 79, 2235; and for (f) and analogues: Müller K.; Obrecht, D.; Knierzinger, A.; Stankovic, C; Spiegler, C.; Trzeciak, A.; Englert, G.; Labhardt, A. M.; Schönholzer, P. *Perspectives in Medicinal Chemistry;*Testa, B., Kyburz, E., Fuhrer, W., Gyger, R., Eds.; Verlag Helv. Chim. Acta: Basel, **1993**; pp 513-531); for (g) and (h) and analogues: Müller, K.; Obrecht, D.; Knierzinger, A.; Spiegler, C.; Bannwarth, W.; Trzeciak, A.; Englert, G.; Labhardt, A.; Schönholzer, P. *Perspectives in Medicinal Chemistry*, Editor Testa, B.; Kyburz, E.; Fuhrer, W.; Giger, R., Weinheim, New York, Basel, Cambridge: Verlag Helvetica Chimica Acta, **1993**, 513-531; Bannwarth, W.; Gerber, F.; Grieder, A.; Knierzinger, A.; Müller, K.; Obrecht. D.; Trzeciak, A. *Can. Pat. Appl.* CA2101599].

As stated above, the process of the invention takes advantage of a mixed solid- and solution phase synthetic approach which can be performed in a parallel array of e.g. 24-192, preferably 96, reactions, and provides the template-fixed cyclic peptides of general formula I in good yields and defined purities, ready for screening, thereby minimizing the amount of dimeric and polymeric impurities, which tend to give false positive hits in the screening process. This process is clearly superior to previously described syntheses of cyclic peptides by Bannwarth, W.; Gerber, F.; Grieder, A.; Knierzinger, A.; Müller, K.; Obrecht. D.; Trzeciak, A. *Can. Pat. Appl.* CA2101599. The proper choice of resin and loading capacity, linker molecule, template and site of cyclization are key for obtaining high yields and reliable purities of β-hairpin loop mimetics. The templates thereby do not only stabilise the conformations of the final products, but they significantly enhance the rate of cyclization to the monomer, most probably by β-hairpin type H-bond induction.

Due to the well-defined architecture of the various β-hairpin loop mimetics of general formula I key amino acid residues and motifs can be locked in various conformations by shifting the sequence along the β-hairpin backbone ("conformational scanning of biologically active sequences"). Alternatively, protein sequences can be mapped by using this approach in order to detect β-hairpin conformations. Thus, this β-hairpin mimetics approach provides a technique to detect hot spots of high energy interactions in protein interfaces in three-dimensional arrangement. This information should ultimately be transferable into the design of small peptidomimetic molecules.

As used in the present description, the term "lower alkyl", taken alone or in combinations such as "aryl-lower alkyl", embraces straight chain or branched saturated hydrocarbon residues with up to 7, preferably up to 4 carbon atoms such as methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec.-butyl, tert.-butyl and the like. The term "lower alkoxy" embraces alkyloxy groups in the sense of the above description of the term "lower alkyl", such as methoxy, ethoxy, n-butoxy, and the like. The term "aryl" embraces the phenyl residue and substituted phenyl residues, especially mono- or disubstituted phenyl residues, with lower alkyl or lower alkoxy groups or halogen atoms primarily coming into consideration as substituents. The term "halogen" denotes the four forms fluorine, chlorine, bromine and iodine unless indicated otherwise. The term "acyl" embraces residues of aliphatic and aromatic carboxylic acids, primarily on the one hand lower alkanoyl groups such as acetyl, propionyl, butyryl and the like, which can be substituted, for example by carboxy or lower alkoxycarbonyl, as is the case e.g. in 4-carboxybutyryl, 4-methoxycarbonylbutyryl or the like, and on the other hand aroyl groups such as the benzoyl group and substituted benzoyl groups, especially mono- or disubstituted benzoyl groups, with lower alkyl or alkoxy groups or halogen atoms primarily coming into consideration as substituents. The term "substituted lower alkyl" embraces lower alkyl groups which are substituted by protected amino, lower alkoxy, $COOR^{10}$ (in which R^{10} is as above), carboxamido or N-lower alkylcarboxamido such as phthalimidomethyl, methoxymethyl, methoxyethyl and the like. The term "protected amino" embraces on the one hand residues such as phthalimido ("Pt") and the like and on the other hand residues of the formula -NH-R^{11} in which R^{11} can signify any appropriate N-protecting group such as benzyloxycarbonyl ("Z"), tert.-butyloxycarbonyl ("Boc"), 9-fluorenylmethoxycarbonyl ("Fmoc"), allyloxycarbonyl ("Alloc"),

trimethylsilylethoxycarbonyl ("Teoc"), trichloroethoxycarbonyl ("Tcc"), o-nitrophenylsulfonyl ("Nps") and the like.

As amino acid residues there primarily come into consideration those which are derived from natural α-amino acids. Hereinafter there is given a list of such amino acids which, or the residues of which, are suitable for the purposes of the present invention, the abbreviations corresponding to generally adopted usual practice.

Ala	A	L-Alanine
Arg	R	L-Arginine
Asn	N	L-Asparagine
Asp	D	L-Aspartic acid
Cys	C	L-Cysteine
Glu	E	L-Glutamic acid
Gln	Q	L-Glutamine
Gly	G	Glycine
His	H	L-Histidine
Ile	I	L-Isoleucine
Leu	L	L-Leucine
Lys	K	L-Lysine
Met	M	L-Methionine
Phe	F	L-Phenylalanine
Pro	P	L-Proline
Ser	S	L-Serine
Thr	T	L-Threonine
Trp	W	L-Tryptophan
Tyr	Y	L-Tyrosine
Val	V	L-Valine

Other α-amino acids which, or the residues of which, are suitable for the purposes of the present invention include

C_4al	L-3-Cyclobutylalanine
C_5al	L-3-Cyclopentylalanine
C_6al	L-3-Cyclohexylalanine
aIle	L-Alloisoleucine
Nal	L-3-(1-Naphthylalanine)
Nle	L-Norleucine
Nva	L-Norvaline
Orn	L-Ornithine
Orn(CHO)	N^5-Formyl-L-ornithine
L-Phg	L-Phenylglycine
Tza	L-3-(2-Thiazolyl)alanine

It will be appreciated that the compound of the above general formula III, i. e. one of the two building blocks of the template structure corresponding to the above formula (a), is a derivative of L-proline (L-Pro, ^{L}P), whilst the second of these building blocks is a residue of D-proline (D-Pro, ^{D}P).

Preferred values for n, i. e. the number of amino acid residues present in the chain Z, are, in general, 4-16. Particularly preferred values of n are 6, 10 and 14 in case the template structure corresponds to the above formula (b) or (c) or (d), and 4, 5, 6, 8, 12 and 16 in the case of the other template structures, i. e. those corresponding to the above formulae (a), (e), (f), (g) and (h).

Advantageously the chain Z consist of, or contains, a key sequence of two, three, four, five, six or occasionally up to ten amino acid residues, the two terminal members of which are "constant" ("k") whilst any other members are either "constant", too or "variable" ("x"), in all possible combinations or permutations. The two terminal "constant" members can be the same or different, and the same applies to any remaining "constant" and/or to any "variable" members.

Particularly suitable "constant" members ("k") are Trp, Arg, Tyr, Ile, Asp, His, Lys, Glu and Thr, further suitable "constant" members ("k") are Gln, Phe, Met and Ser, and suitable "variable" members ("x") are Ala, Orn, Leu and Val.

Key sequences of two, three, four, five and six amino acid residues, can be schematically depicted as follows:

dipeptide

$-k^1-k^2-$

tripeptide

$-k^1-k^2-k^3-$

$-k^1-x^1-k^2-$

tetrapeptide

$-k^1-k^2-k^3-k^4-$

$-k^1-x^1-k^2-k^3-$

$-k^1-k^2-x^1-k^3-$

$-k^1-x^1-x^2-k^2-$

pentapeptide

$-k^1-k^2-k^3-k^4-k^5-$

$-k^1-x^1-k^2-k^3-k^4-$

$-k^1-k^2-x^1-k^3-k^4-$

$-k^1-k^2-k^3-x^1-k^4-$

$-k^1-x^1-x^2-k^2-k^3-$

$-k^1-k^2-x^1-x^2-k^3-$

$-k^1-x^1-k^2-x^2-k^3-$

$-k^1-x^1-x^2-x^3-k^2-$

hexapeptide

$-k^1-k^2-k^3-k^4-k^5-k^6-$

$-k^1-x^1-k^2-k^3-k^4-k^5-$

$-k^1-k^2-x^1-k^3-k^4-k^5-$

$-k^1-k^2-k^3-x^1-k^4-k^5-$

$-k^1-k^2-k^3-k^4-x^1-k^5-$

$-k^1-x^1-x^2-k^2-k^3-k^4-$

$-k^1-k^2-x^1-x^2-k^3-k^4-$
$-k^1-k^2-k^3-x^1-x^2-k^4-$
$-k^1-x^1-k^2-x^2-k^3-k^4-$
$-k^1-k^2-x^1-k^3-x^2-k^4-$
$-k^1-x^1-k^2-k^3-x^2-k^4-$
$-k^1-x^1-x^2-x^3-k^2-k^3-$
$-k^1-k^2-x^1-x^2-x^3-k^3-$
$-k^1-x^1-k^2-x^2-x^3-k^3-$
$-k^1-x^1-x^2-k^2-x^3-k^3-$
$-k^1-x^1-x^2-x^3-x^4-k^2-$

Certain key sequences are known to occur in important physiologically active peptides, such as

R G D — in fibronectin (FN), vitronectin (VN), osteopontin, collagens, thrombospondin, fibrinogen (Fg), von Willebrand factor (vWF), see Obrecht, D.; Altorfer, M.; Robinson, J. A. *Adv. Med. Chem.* Vol. *4*, 1-68, JAI Press Inc., **1999**

E L R — in C X C chemokines, see Saunders, J.; Tarby, C. M. *Drug Discovery Today*, **1999**, *4*, 80-92

R K K — see *J. Biol. Chem.* **1999**, *274*, 3513

K G F — see *Prot. Sci.* **1998**, *7*, 1681-1690

V R K K — [SEQ ID NO:1] in Platelet-Derived Growth Factor (PDGF), see Ross, R.; Raines, E. W.; Bowden-Pope, D. F. *Cell*, **1986**, *46*, 155-159

K K Y L — [SEQ ID NO:2] in VIP (vasointestinal peptide) showing neuroprotective properties against β-amyloid neurotoxicity, see *Proc. Natl. Am. Soc. USA* **1999**, *96*, 4143-4148

W L D V [SEQ ID NO:3] in integrin $\alpha_4\beta_1$, see *Europ. J. Biol.* **1996**, *242*, 352-362 and *Int. J. Pept. Prot. Res.* **1996**, *47*, 427-436

Y I R L P [SEQ ID NO:4] in Factor Xa inhibitors, see Al Obeidis,F.; Ostrem, J. A. *Drug Discovery Today* **1998** , *3*, 223-231

Y I G S R [SEQ ID NO:5] in laminine, see *EMBO. J.* **1984**, *3*, 1463

I K V A V [SEQ ID NO:6] see *Cell* **1987**, *88*, 989

P P R X X W [SEQ ID NO:7] see *J. Biol. Chem.* **1998**, *273*, 11001-11006 & 11007-11011

I Y Y K D G A L K Y [SEQ ID NO:8] see *Biochem Soc. Trans.* **1997**, *29*, 387-392

If desired, the process of the invention can be modified to give the enantiomers of the compounds of the general formula I. To this effect all amino acids which have an asymmetric α-carbon atom are used in their D-Form and the enantiomer of a template corresponding to structure (a), (b), (c), (d) or (e) or a template corresponding to formula (f), (g) or (h) is used in step (f) and, respectively, the enantiomer of a compound of formula III is used in step (fa) and a derivative of L-proline is used in step (fc).

Suitable protecting groups for amino acids and, respectively, for their residues are, for example,

- for the amino group (as is present e. g. also in the side-chain of lysine)

Z	benzyloxycarbonyl
Boc	tert.-butyloxycarbonyl
Fmoc	9-fluorenylmethoxycarbonyl
Alloc	allyloxycarbonyl
Teoc	trimethylsilylethoxycarbonyl
Tcc	trichloroethoxycarbonyl

Nps	o-nitrophenylsulfonyl;
Tr	triphenymethyl or trityl

- for the carboxyl group (as is present e. g. also in the side-chain of aspartic and glutamic acid) by conversion into esters with the alcohol components

tBu	tert.-butyl
Bn	benzyl
Me	methyl
Ph	phenyl
Pac	Phenacyl
	Allyl
	trimethylsilylethyl
	trichloroethyl;

- for the guanidino group as is present e. g. in the side-chain of arginine)

Pmc	2,2,5,7,8-pentamethylchroman-6-sulfonyl
Ts	tosyl (i. e. p-toluenesulfonyl)
Z	benzyloxycarbonyl
Pbf	pentamethyldihydrobenzofuran-5-sulfonyl

- for the hydroxy group (as is present e. g. in the side-chain of threonine and serine)

tBu	tert.-butyl
Bn	benzyl
Tr	trityl

- and for the mercapto group (as is present e. g. in the side-chain of cysteine)

tBu	tert.-butyl
Bn	benzyl

Tr	trityl
Mtr	2-methoxytrityl.

The functionalized solid support is conveniently derived from polystyrene crosslinked with, preferably 1-5%, divinylbenzene; polystyrene coated with polyethyleneglycol spacers (Tentagel^{R}); and polyacrylamide resins (see also Obrecht, D.; Villalgordo, J.-M, "Solid-Supported Combinatorial and Parallel Synthesis of Small-Molecular-Weight Compound Libraries", *Tetrahedron Organic Chemistry Series*, Vol. 17, Pergamon, Elsevier Science, **1998**).

The solid support is functionalized by means of a linker, i.e. a bifunctional spacer molecule which contains on one end an anchoring group for attachment to the solid support and on the other end a selectively cleavable functional group used for the subsequent chemical transformations and cleavage procedures. For the purposes of the present invention the linker must be designed to eventually release the carboxyl group under mild acidic conditions which do not affect protecting groups present on any functional group in the side-chains of the various amino acids. Linkers which are suitable for the purposes of the present invention form acid-labile esters with the carboxyl group of the amino acids, usually acid-labile benzyl, benzhydryl and trityl esters; examples of linker structures of this kind include 3-methoxy-4-hydroxymethylphenoxy (Sasrin linker), 4-(2,4-dimethoxyphenyl-hydroxymethyl)-phenoxy (Rink linker), 4-(4-hydroxymethyl-3-methoxyphenoxy)butyric acid (HMPB linker), trityl and 2-chlorotrityl.

When carried out as a parallel array synthesis the process of the invention can be advantageously carried out as described hereinbelow but it will be immediately apparent to those skilled in the art how this procedure will have to be modified in case it is desired to synthesize one single compound of the above formula I.

A number of reaction vessels (normally 24 to 192, typically 96) equal to the total number of compounds to be synthesized by the parallel method are loaded with 25 to 1000 mg, preferably 100 mg, of the appropriate functionalized solid support, preferably 1 to 3% cross linked polystyrene or tentagel resin.

The solvent to be used must be capable of swelling the resin and includes, but is not limited to, dichloromethane (DCM), dimethylformamide (DMF), N-methylpyrrolidinone (NMP), dioxane, toluene, tetrahydrofuran (THF), ethanol (EtOH), trifluoroethanol (TFE), isopropylalcohol and the like. Solvent mixtures containing as at least one component a polar solvent (e. g. 20% TFE/DCM, 35% THF/NMP) are beneficial for ensuring high reactivity and solvation of the resin-bound peptide chains (Fields, G. B., Fields, C. G., *J. Am. Chem. Soc.* **1991**, *113*, 4202-4207).

With the development of various linkers that release the C-terminal carboxylic acid group under mild acidic conditions, not affecting acid-labile groups protecting functional groups in the side chain(s), considerable progresses have been made in the synthesis of protected peptide fragments. The 2-methoxy-4-hydroxybenzylalcohol-derived linker (Sasrin^R linker, Mergler et al., *Tetrahedron Lett.* **1988**, 29 4005-4008) is cleavable with diluted trifluoroacetic acid (0.5-1% TFA in DCM) and is stable to Fmoc deprotection conditions during the peptide synthesis, Boc/tBu-based additional protecting groups being compatible with this protection scheme. Other linkers which are suitable for the process of the invention include the super acid labile 4-(2,4-dimethoxyphenyl-hydroxymethyl)-phenoxy linker (Rink linker, Rink, H. *Tetrahedron Lett.* **1987**, *28*, 3787-3790), where the removal of the peptide requires 10% acetic acid in DCM or 0.2% trifluoroacetic acid in DCM; the 4-(4-hydroxymethyl-3-methoxyphenoxy)butyric acid-derived linker (HMPB- linker, Flörsheimer & Riniker, *Peptides* **1991**,1990 131) which is also cleaved with 1%TFA/DCM in order to yield a peptide fragment containing all acid labile side- chain protective groups; and, in addition, the 2-chlorotritylchloride linker (Barlos et al., *Tetrahedron Lett.* **1989**, *30*, 3943-3946), which allows the peptide detachment using a mixture of glacial acetic acid/trifluoroethanol/DCM (1:2:7) for 30 min.

The 9-fluorenylmethoxycarbonyl- (Fmoc)-protected amino acid derivatives are preferably used as the building blocks for the construction of the template-fixed β-hairpin loop mimetics of formula I. For the deprotection, i. e. cleaving off of the Fmoc group, 20% piperidine in DMF or 2% DBU/2% piperidine in DMF can be used.

The quantity of the reactant, i. e. of the amino acid derivative, is usually 1 to 20 equivalents based on the milliequivalents per gram (meq/g) loading of the functionalized solid support

(typically 0.1 to 2.85 meq/g for polystyrene resins) originally weighed into the reaction tube. Additional equivalents of reactants can be used if required to drive the reaction to completion in a reasonable time. The reaction tubes, in combination with the holder block and the manifold, are reinserted into the reservoir block and the apparatus is fastened together. Gas flow through the manifold is initiated to provide a controlled environment, for example, nitrogen, argon, air and the like. The gas flow may also be heated or chilled prior to flow through the manifold. Heating or cooling of the reaction wells is achieved by heating the reaction block or cooling externally with isopropanol/dry ice and the like to bring about the desired synthetic reactions. Agitation is achieved by shaking or magnetic stirring (within the reaction tube). The preferred workstations (without, however, being limited thereto) are Labsource's Combi-chem station and MultiSyn Tech's-Syro synthesizer.

Amide bond formation requires the activation of the α-carboxyl group for the acylation step. When this activation is being carried out by means of the commonly used carbodiimides such as dicyclohexylcarbodiimide (DCC, Sheehan & Hess, *J. Am. Chem. Soc.* **1955,** *77*, 1067-1068) or diisopropylcarbodiimide (DIC, Sarantakis et al *Biochem. Biophys. Res. Commun.***1976**, *73*, 336-342), the resulting dicyclohexylurea is insoluble and, respectively, diisopropylurea is soluble in the solvents generally used. In a variation of the carbodiimide method 1-hydroxybenzotriazole (HOBt, König & Geiger, *Chem. Ber* **1970**, *103*, 788-798) is included as an additive to the coupling mixture. HOBt prevents dehydration, suppresses racemization of the activated amino acids and acts as a catalyst to improve the sluggish coupling reactions. Certain phosphonium reagents have been used as direct coupling reagents, such as benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate (BOP) (Castro et al., *Tetrahedron Lett.* **1975**, *14,* 1219-1222; *Synthesis*, **1976**, 751-752), or benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexaflurophoshate (Py-BOP, Coste et al., *Tetrahedron Lett.* **1990**, *31*, 205-208), or 2-(1H-benzotriazole-1-yl-)1,1,3,3-tetramethyluronium terafluoroborate (TBTU), or hexaflurorophosphate (HBTU, Knorr et al., *Tetrahedron Lett.* **1989**, *30*, 1927-1930); these phosphonium reagents are also suitable for in situ formation of HOBt esters with the protected amino acid derivatives. More recently diphenoxyphosphoryl azide (DPPA) or O-(7-aza-benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate (TATU) or O-(7-aza-benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HATU)/7-aza-1-

hydroxy benzotriazole (HOAt, Carpino et al., *Tetrahedron Lett.* **1994**, *35*, 2279-2281) have also been used as coupling reagents.

Due to the fact that near-quantitative coupling reactions are essential it is desirable to have experimental evidence for completion of the reactions. The ninhydrin test (Kaiser et al., *Anal. Biochemistry* **1970**, *34*, 595), where a positive colorimetric response to an aliquot of resin-bound peptide indicates qualitatively the presence of the primary amine, can be easily and quickly performed after each coupling step. Fmoc chemistry allows the spectrophotometric detection of the Fmoc chromophore when it is released with the base (Meienhofer et al., *Int. J. Peptide Protein Res.* **1979**, *13*, 35-42).

The resin-bound intermediate within each reaction tube is washed clean of excess of retained reagents, of solvents, and of by-products by repetitive exposure to clean solvent(s) by one of the two following methods:

1) The reaction wells are filled with solvent (preferably 5 ml), the reaction tubes, in combination with the holder block and manifold, are immersed and agitated for 5 to 300 minutes, preferably 15 minutes, and drained by gravity followed by gas pressure applied through the manifold inlet (while closing the outlet) to expel the solvent;

2) The manifold is removed from the holder block, aliquots of solvent (preferably 5 ml) are dispensed through the top of the reaction tubes and drained by gravity through a filter into a receiving vessel such as a test tube or vial.

Both of the above washing procedures are repeated up to about 50 times (preferably about 10 times), monitoring the efficiency of reagent, solvent, and byproduct removal by methods such as TLC, GC, or visualization of the wash filtrates.

The above described procedure of reacting the resin-bound compound with reagents within the reaction wells followed by removal of excess reagents, by-products, and solvents is repeated with each successive transformation until the final resin-bound compound is prepared.

Detachment of the fully protected linear peptide from the solid support is achieved by immersion of the reaction tubes, in combination with the holder block and manifold, in reaction wells containing a solution of the cleavage reagent (preferably 3 to 5 ml). Gas flow, temperature control, agitation, and reaction monitoring are implemented as described above and as desired to effect the detachment reaction. The reaction tubes, in combination with the holder block and manifold, are disassembled from the reservoir block and raised above the solution level but below the upper lip of the reaction wells, and gas pressure is applied through the manifold inlet (while closing the outlet) to efficiently expel the final product solution into the reservoir wells. The resin remaining in the reaction tubes is then washed 2 to 5 times as above with 3 to 5 ml of an appropriate solvent to extract (wash out) as much of the detached product as possible. The product solutions thus obtained are combined, taking care to avoid cross-mixing. The individual solutions/extracts are then manipulated as needed to isolate the final compounds. Typical manipulations include, but are not limited to, evaporation, concentration, liquid/liquid extraction, acidification, basification, neutralization or additional reactions in solution.

The solutions containing fully protected linear peptide derivatives which have been cleaved off from the solid support and neutralized with a base, are evaporated, then cyclization is effected in solution using solvents such as DCM, DMF, Dioxane, THF and the like. Various coupling reagents which were mentioned earlier can be used for the cyclization. The duration of the cyclization is about 6-48 hours, preferably about 24 hours. The progress of the reaction is followed, e. g. by RP-HPLC (Reverse Phase High Performance Liquid Chromatography). Then the solvent is removed by evaporation, the fully protected cyclic peptide derivative is dissolved in a solvent which is not miscible with water, such as DCM, and the solution is extracted with water or a mixture of water-miscible solvents, in order to remove any excess of the coupling reagent.

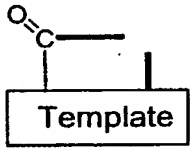
The fully protected cyclized peptide derivative is treated with 95% TFA, 2.5% H_2O, 2.5% TIS or another combination of scavengers for effecting the cleavage of protecting groups. The cleavage reaction time is commonly 30 minutes to 12 hours, preferably 2 hours. Thereafter most of the TFA is evaporated and the product is precipitated with ether/hexane (1:1) or other solvents which are suitable therefor. After careful removal of the solvent, the cyclic peptide derivative obtained as end-product can be isolated. Depending on its purity, this peptide derivative can be

used directly for biological assays, or it has to be further purified, for example by preparative HPLC.

The end-products, i. e. the compounds of formula I, can be individually tested for biological activity once they have been isolated and characterized. For example, the following Solid-Phase assay can be carried out.

Direct immobilization of platelet-derived growth factor β (PDGFR-β) is performed by overnight incubation in immunosorbent 96-well plates (Nunc) at 4°C using 100ng of purified protein in 100µl of 15mM Na_2CO_3, 35 mM $NaHCO_3$, pH 9.6. The plates are washed once with tris-buffered saline (TBS, 20mM Tris-HCl, 150mM NaCl, pH 7.4), and nonspecific adsorption is blocked by at least 1h of incubation with TBS plus 1 % bovine serum albumin (BSA). Following washing with TBS plus 0.1% Tween, 3000 cpm of ^{125}I-PDGF-BB and increasing amounts of unlabeled PDGF-BB or the peptide derivative of formula I are added to duplicate wells and incubated for 3h at room temperature in 0.1% Tween, 1mM $CaCl_2$, 1mM $MgCl_2$ and 1%BSA. The plates are washed three times with TBS plus 0.1%Tween, and bound ligand is removed with 0.1M citric acid, pH 2.5, prior to counting in a γ-counter.

Some of the compounds embraced by general formula I have already been described but the remaining of these compounds are novel and form part of the present invention, namely those of formula I with the provisos that if



is

(i) group (a) and R^1 is hydrogen, then Z is other than

-Val-Lys-Asn-Tyr-Gly-Val-Lys-Asn-Ser-Glu-Trp-Ile- [SEQ ID NO:9],

-Val-Lys-Asn-Tyr-Gly-Val-Lys-Asn-Ser-Glu-Trp-Thr- [SEQ ID NO:10],

-Gly-Arg-Gly-Asp- [SEQ ID NO:11],

-Arg-Gly-Asp-Gly- [SEQ ID NO:12],

-Phe-Tyr-Thr-Gly-Thr- [SEQ ID NO:13],
-Tyr-Arg-Asp-Ala-Met- [SEQ ID NO:14],
-Asn-Thr-Tyr-Ser-Gly-Val- [SEQ ID NO:15],
-Trp-Asp-Asp-Gly-Ser-Asp- [SEQ ID NO:16] and
-Leu-Trp-Tyr-Ser-Asn-His-Trp-Val- [SEQ ID NO:17];

(ii) group (b) and R^2 is hydrogen or CH_2-COOH, or group (c) and R^3 is benzoyl, or group (d), or group (e), then Z is other than -Ala-Asn-Pro-Asn-Ala-Ala- [SEQ ID NO:18];

(iii) group (b) and R^2 is hydrogen, then Z is other than -Ala-Arg-Gly-Asp- [SEQ ID NO:19];

(iv) group (f), R^4 is methyl, R^5 is methoxy and R^6 and R^7 each are hydrogen, then Z is other than

-Val-Ala-Ala-Phe-Leu-Ala-Leu-Ala- [SEQ ID NO:20],
-Arg-Gly-Asp-Val- [SEQ ID NO:21],
-Ala-Thr-Val-Gly- [SEQ ID NO:22],
-Glu-Arg-Gly-Asp-Val-Tyr- [SEQ ID NO:23],
-Ile-Ala-Arg-Gly-Asp-Phe-Pro-Asp- [SEQ ID NO:24],
-Ala-Arg-Ile-Ala-Arg-Gly-Asp-Phe-Pro-Asp-Asp-Arg- [SEQ ID NO:25],
-Ala-Arg-Gly-Asp-Phe-Pro- [SEQ ID NO:26],
-Arg-Gly-Asp-Phe- [SEQ ID NO:27] and
-Arg-Ile-Ala-Arg-Gly-Asp-Phe-Pro-Asp-Asp- [SEQ ID NO:28];

(v) group (g), R^8 is methyl and R^9 is methyl or n-hexyl, or group (h), R^8 is ethyl and R^9 is ethyl, then Z is other than -Arg-Gly-Asp-Val- [SEQ ID NO:21];

(vi) group (g), R^8 is methyl and R^9 is methyl or benzyl, then Z is other than -Gly-Gly-Ala-Gly- [SEQ ID NO:29];

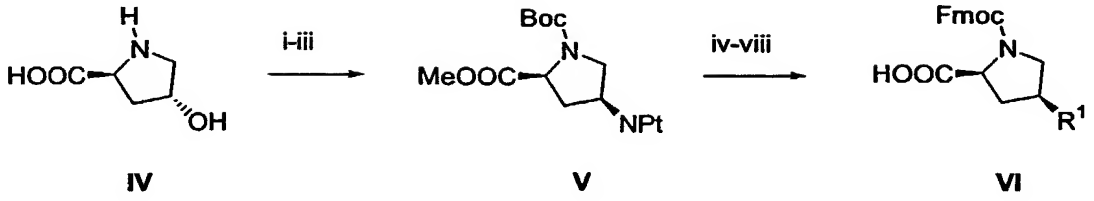
(vii) group (g), R^8 is methyl and R^9 is methyl, then Z is other than -Gly-Asp-Gly-Gly- [SEQ ID NO:30]; and

(viii) group (g), R^8 is methyl and R^9 is n-hexyl, then Z is other than -Val-Arg-Lys-Lys- [SEQ ID NO:1].

The enantiomers of all compounds of formula I are novel and also form part of the present invention.

The compounds of formula II incorporating structures (a) to (h) and the compounds of formula III can be prepared as shown in the following Reaction Schemes. Throughout these Reaction Schemes the N-protecting group X present in the compounds of formulae II and III is indicated to be Fmoc, the preferred value for X, but it will be appreciated that corresponding compounds carrying as X other N-protecting groups can be prepared in a similar way.

Reaction Scheme 1



IV V VI

IV ⟶ V

i: Treatment of **IV** with a dehydrating reagent such as thionylchloride in methanol at an elevated temperature, conveniently at reflux.

ii: Introduction of Boc, e.g. using di-tert.-butyl dicarbonate and triethylamine in a suitable solvent such as dichloromethane; any other suitable N-protecting group (not shown in Reaction Scheme 1) can be introduced in an analogous manner.

iii: Reaction of formed product with phthalimide, diethyl diazodicarboxylate and triphenylphoshine under standard Mitsunobu conditions (Mitsunobu, O.; Wada, M.; Sano, T. J. *J. Am. Chem. Soc.* **1972**, *94*, 672) to conveniently yield **V**.

V ⟶ VI

iv: Cleavage of the phthalimide group, suitably by treatment of **V** with hydrazine hydrate in a suitable solvent, such as ethanol, at an elevated temperature, conveniently at about 80° C.

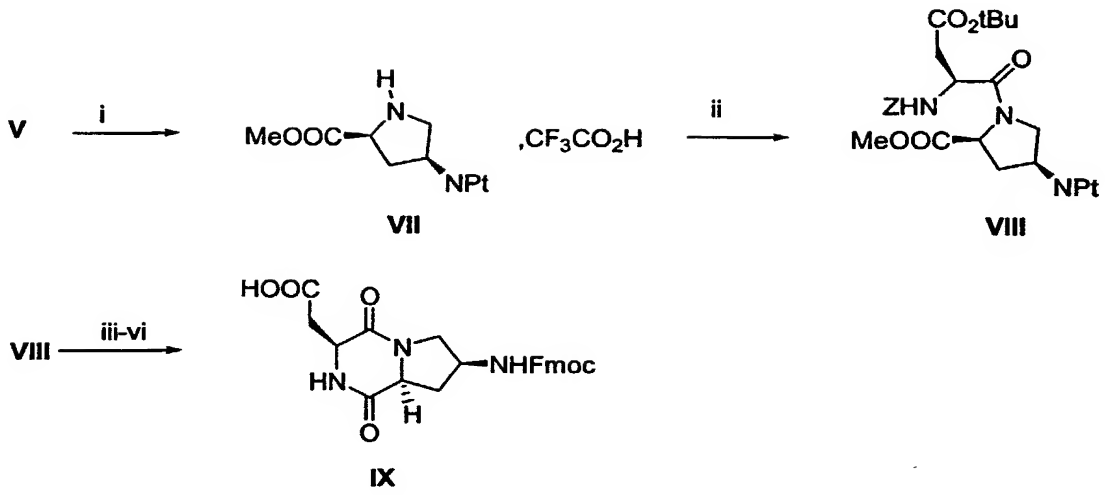
v: Standard protection of the 3-amino group.

vi: Saponification of the methyl ester group using e.g. a suitable basic reagent such as lithium hydroxide in methanol and water.

vii: The tert.-butoxycarbonyl group is subsequently cleaved off using reagents such as trifluoroacetic acid in dichloromethane or 4N hydrochloric acid in dioxane.

viii: The formed amino acid is conveniently protected with reagents such as 9-fluorenylmethoxcarbonyl chloride or 9-fluorenylmethoxcarbonyl succinimide using a base such as sodium carbonate or triethylamine in a suitable solvent or mixture of solvents such as dioxane and water, or dichloromethane to yield **VI**.

Reaction Scheme 2



V ——————→ VII

i: Treatment of V with trifluoracetic acid in dichloromethane.

VII ——————→ VIII

ii: **VII** is coupled under standard peptide coupling conditions with Z-Asp(tBu)OH in DMF with reagents such as HBTU and 1-hydroxybenztriazole (HOBt) with a base such as diisopropylethylamine to yield **VIII**.

VIII ——————→ IX

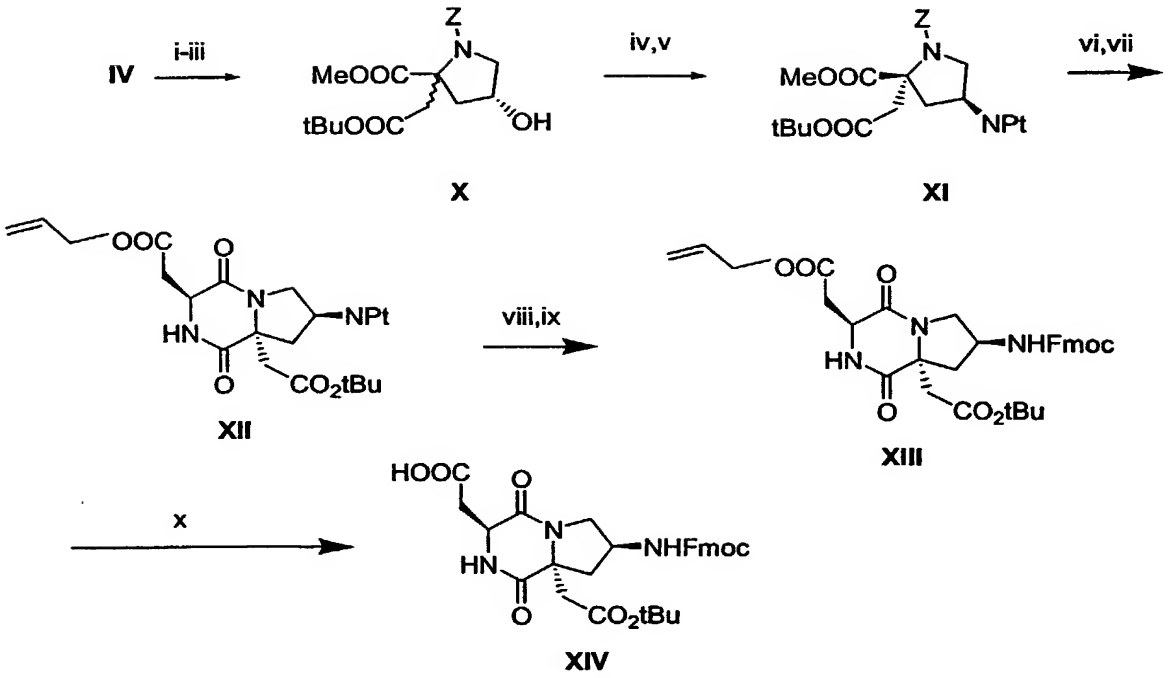
iii: Removal of the Z-group, conveniently by hydrogenation using H_2 and a catalyst such as Palladium on charcoal, in solvents such as ethanol, DMF and ethyl acetate.

iv: The phthalimide group is cleaved off from the resulting product, conveniently by treatment with hydrazine in a suitable solvent such as ethanol at an elevated temperature, suitably at about 80° C.

v: The formed amino acid is conveniently protected with reagents such as 9-fluorenylmethoxcarbonyl chloride or 9-fluorenylmethoxcarbonyl succinimide using a

base such as sodium carbonate or triethylamine in a suitable solvent or mixture of solvents such as dioxane and water, or dichloromethane to yield **IX** as described by Bisang, C.; Weber, C.; Robinson, J. A. *Helv. Chim. Acta* **1996**, *79*, 1825-1842.

Reaction Scheme 3



IV ——————→ X

i: Treatment of **IV** with a dehydrating reagent such as thionyl chloride in a suitable solvent such as methanol at an elevated temperature, conveniently at reflux.

ii: The resulting amino acid ester is N-protected under standard conditions for introducing the Z group, e.g. using benzyloxycarbonyl chloride and triethylamine in a suitable solvent such as dichloromethane.

iii: The Z-protected amino acid methyl ester is treated with trimethylsilylchloride and a base such as triethylamine in a solvent such as tetrahydrofuran, cooled, conveniently to about –78° C, followed by reaction with a strong base such as lithium diisopropylamide or lithium hexamethyldisilylazide and tert.-butyl bromoacetate yielding **X** as a mixture of diastereomers as described by Bisang, C.; Jiang, L.; Freund, E.; Emery, F.; Bauch, C.; Matile, H,; Pluschke, G.; Robinson, J. A. *J. Am. Chem. Soc.* **1998**, *120*, 7439-7449;

Emery, F.; Bisang, C.; Favre, M.; Jiang, L.; Robinson, J. A. *J. Chem. Soc. Chem. Commun.* **1996**, 2155-2156.

X ——————→ XI

iv: Reaction of X with phthalimide, diethyl diazadicarboxylate and triphenylphoshine under standard Mitsunobu conditions (Mitsunobu, O.; Wada, M.; Sano, T. J. *J. Am. Chem. Soc.* **1972**, *94*, 672.

v: The resulting product is hydrogenated using H_2 and a suitable catalyst such as Palladium on charcoal in a solvent such as ethyl acetate, DMF or ethanol; subsequently separation of diastereomers takes place.

XI ——————→ XII

vi: XII is coupled with Fmoc-Asp(allyl)OH under standard peptide coupling conditions using reagents such as HATU, HOAt and a base such as diisopropylethylamine in a suitable solvent such as DMF.

vii: Cyclization, conveniently with DBU in DMF.

XII ——————→ XIII

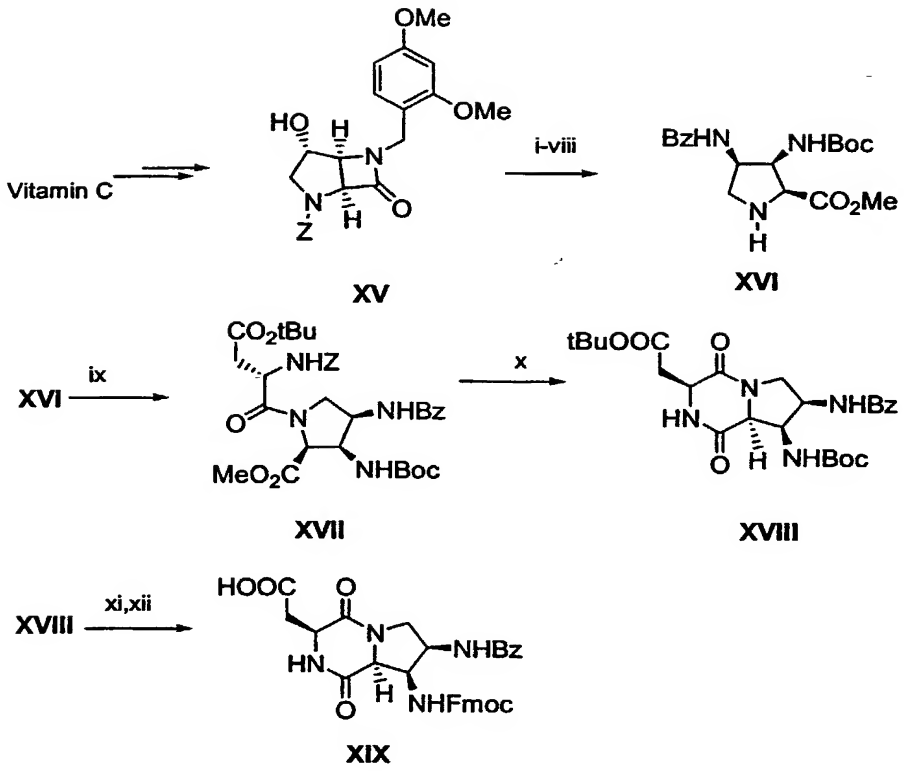
viii: The phthalimide group is cleaved off from resulting product, conveniently by hydrazinolysis, e.g. treatment with methylhydrazine in a suitable solvent such as DMF.

ix: The formed product is conveniently protected with reagents such as 9-fluorenylmethoxcarbonyl chloride or 9-fluorenylmethoxcarbonyl succinimide using a base such as sodium carbonate or triethylamine in a suitable solvent or mixture of solvents such as dioxane and water, or dichloromethane.

XIII ——————→ XIV

x: Standard removal of an allyl ester group using e.g. Palladium(0) as catalyst.

Reaction Scheme 4



XV ⟶ XVI

i : XV (obtainable from Vitamin C as described by Hubschwerlen, C. (*Synthesis* **1986**, 962) is treated with phthalimide, diethyl diazodicarboxylate and triphenylphoshine under standard Mitsunobu conditions (Mitsunobu, O.; Wada, M.; Sano, T. J. *J. Am. Chem. Soc.* **1972**, *94*, 672).

ii: The phthalimide group is cleaved off from the product, conveniently by hydrazinolysis, e.g. by treatment with methylhydrazine in a suitable solvent such as DMF.

iii: The amino group is protected by treatment with a benzoylating reagent such as benzoic acid anhydride or benzoylchloride and a base such as triethylamine or 4-dimethylaminopyridine in a suitable solvent such as dichloromethane or DMF.

iv: Removal of the 2,4-dimethoxybenzyl group, e.g. with $K_2S_2O_8$ and Na_2HPO_4 in aqueous acetonitrile at an elevated temperature, e.g. at about 80° C.

v: Introduction of a tert.-butoxycarbonyl group using e.g. di-tert.-butyloxycarbonyl dicarbonate, triethylamine and a catalytic amount of 4-dimethylaminopyridin in a suitable solvent such as dichloromethane.

vi: Reaction with aqueous sodium carbonate in tetrahydrofuran followed by acidification.

vii: Esterification of the carboxylic acid group, conveniently with diazomethane in a suitable solvent such as diethylether.

viii Removal of the Z-group, conveniently by hydrogenation with H_2 in the presence of a catalyst such as Palladium on charcoal in a solvent such as DMF to yield **XVI** as described by Pfeifer, M.; Robinson, J. A. *J. Chem. Soc. Chem. Commun.* **1998**, 1977.

XVI ⟶ **XVII**

ix: **XVI** is coupled under standard peptide coupling conditions with Z-Asp(tBu)OH in DMF with reagents such as HBTU and 1-hydroxybenztriazole with a base such as diisopropylethylamine to yield **XVII** as described by Pfeifer, M.; Robinson, J. A. *J. Chem. Soc. Chem. Commun.* **1998**, 1977.

XVII ⟶ **XVIII**

x: Removal of the Z-group, e.g. by hydrogenation using H_2 and a catalyst such as Palladium on charcoal under standard conditions, yields **XVIII** as described by Pfeifer, M.; Robinson, J. A. *J. Chem. Soc. Chem. Commun.* **1998**, 1977.

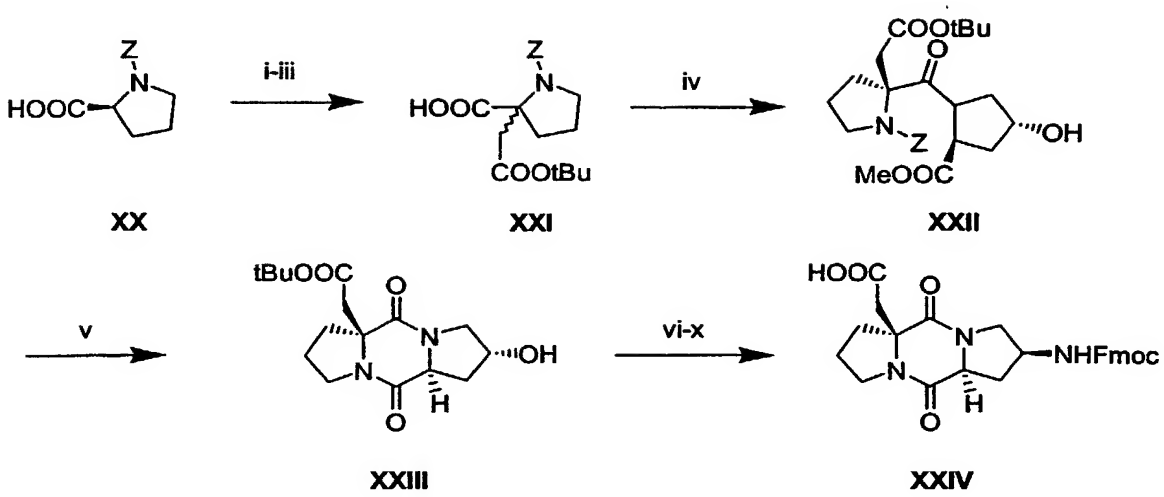
XVIII ⟶ **XIX**

xi: Cleavage of the tert.-butyl ester and tert.-butyloxycarbonyl groups, conveniently using trifluoracetic acid in dichloromethane or 4N hydrochloric acid in dioxane.

xii: The intermediate free amino acid formed is conveniently protected with reagents such as 9-fluorenylmethoxcarbonyl chloride or 9-fluorenylmethoxcarbonyl succinimide using a base such as sodium carbonate or triethylamine in a suitable solvent or mixture of

solvents such as dioxane and water, or dichloromethane to yield **XIX** as described by Pfeifer, M.; Robinson, J. A. *J. Chem. Soc. Chem. Commun.* **1998**, 1977.

Reaction Scheme 5



XX ——————————→ XXI

i: Treatment of **XX** wit a dehydrating agent such as thionyl chloride in a suitable solvent such as methanol at an elevated temperature, conveniently at about 80° C.

ii: The intermediate is treated with a strong base such as lithium diisopropylamide or lithium hexamethyldisilylazide in a suitable solvent such as tetrahydrofuran at low temperature, and with tert.-butyl bromoacetate as described by Pfeifer, M.; Linden, A.; Robinson, J. A. *Helv. Chim. Acta* **1997**, *80*, 1513-1527.

iii: Saponification using a base such as lithium hydroxide in water and a suitable solvent such as methanol.

XXI ——————————→ XXII

iv: Coupling of **XXI** with (2*S*,4*R*)-Z-hydroxy proline under standard peptide coupling conditions, e.g. using reagents such as HBTU and HOBT and diisopropylethylamine as base in a suitable solvent such as DMF, yielding **XXII** as described by Pfeifer, M.; Linden, A.; Robinson, J. A. *Helv. Chim. Acta* **1997**, *80*, 1513-1527.

XXII ——————————→ XXIII

v: Removal of the Z-group, e.g. by hydrogenation using H_2 and a catalyst such as Palladium on charcoal in a suitable solvent such as ethyl acetate.

XXIII ——————————→ XXIV

vi: **XXIII** is converted into the corresponding tosylate according to standard methods, e.g. by reaction with p-toluenesulfonyl chloride in pyridine.

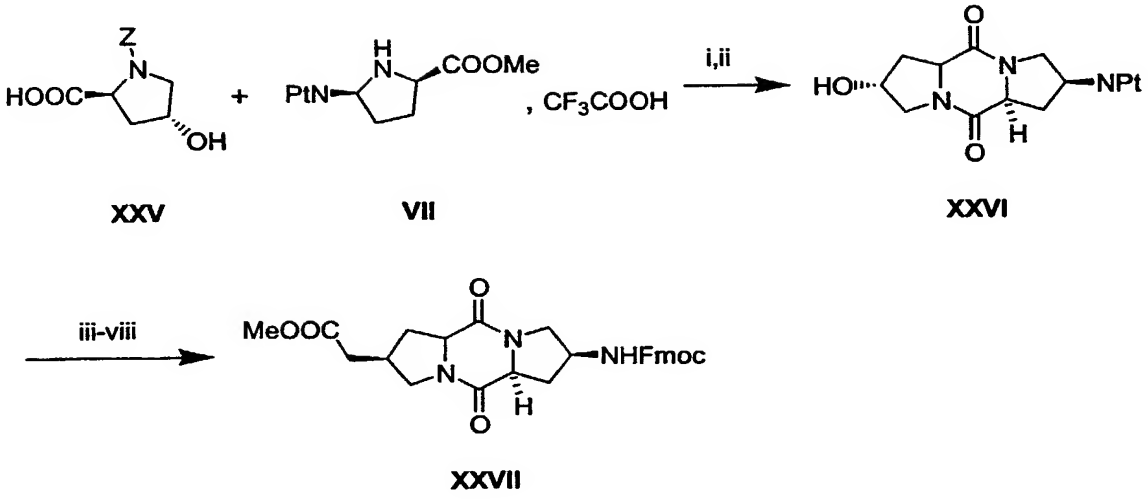
vii: The intermediate tosylate is converted into the corresponding azide, e.g. by treatment with sodium azide in a suitable solvent such as DMF at an elevated temperature, conveniently at about 80° C.

viii: Reduction of the azide group to the amino group can conveniently be performed with H_2 and a catalyst such as Palladium on charcoal in a suitable solvent such as ethyl acetate, or with triphenylphoshine.

ix: The intermediate free amino acid tert.-butylester is conveniently protected with reagents such as 9-fluorenylmethoxcarbonyl chloride or 9-fluorenylmethoxcarbonyl succinimide using a base such as sodium carbonate or triethylamine in a suitable solvent or mixture of solvents such as dioxane and water, or dichloromethane.

x: Acidolysis using e.g. trifluoracetic acid in dichloromethane gives conveniently **XXIV** as described by Pfeifer, M.; Linden, A.; Robinson, J. A. *Helv. Chim. Acta* **1997**, *80*, 1513-1527.

Reaction Scheme 6



XXV ⟶ XXVI

i: Standard peptide coupling of **VII** with **XXV** under standard peptide coupling conditions using reagent such as HBTU and HOBT and e.g. diisopropylethylamine as base in a suitable solvent such as DMF.

ii: Hydrogenation using H_2 and a catalyst such as Palladium on charcoal in solvents such as ethyl acetate, DMF and ethanol yields **XXVI** as described by Beeli, R.; Steger, M.; Linden, A.; Robinson, J. A. *Helv. Chim. Acta* **1996**, *79*, 2235-2248.

XXVI ⟶ XXVII

iii: Oxidation of the OH group using reagents such as pyridine-sulfur trioxide complex, Jones reagent or the Dess-Martin periodinane reagent.

iv: Wittig-Horner condensation of the intermediate ketone with $(MeO)_2POCH_2COOMe$ and a base such as sodium hexamethyldisilylazide in solvents such as tetrahydrofuran or dimethoxyethane as described by Beeli, R.; Steger, M.; Linden, A.; Robinson, J. A. *Helv. Chim. Acta* **1996**, *79*, 2235-2248.

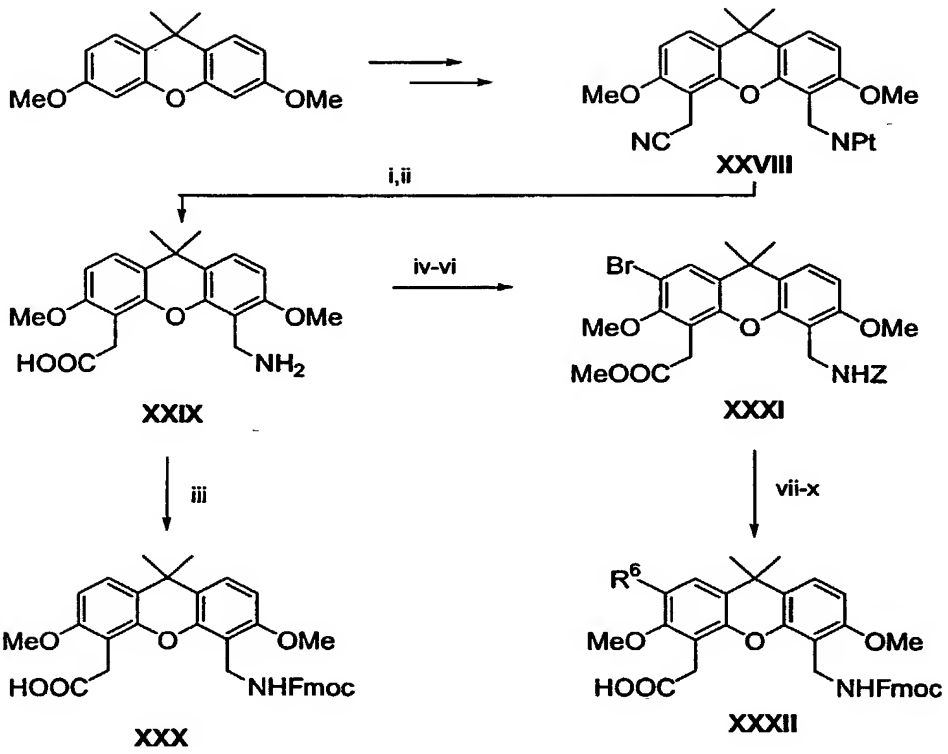
v: Stereoselective hydrogenation of the double bond using e.g. H_2 and a catalyst such as Palladium on charcoal in a solvent such as ethanol, DMF and ethyl acetate.

vi: Hydrazinolysis of the intermediate phthalimide using e.g. hydrazine in a suitable solvent such as ethanol at an elevated temperature, conveniently at about 80° C.

vii: Saponification of the methyl ester group, e.g. by treatment with a suitable basic reagent such as lithium hydroxide in water and methanol.

viii: The intermediate free amino acid formed is conveniently protected with reagents such as 9-fluorenylmethoxcarbonyl chloride or 9-fluorenylmethoxcarbonyl succinimide using a base such as sodium carbonate or triethylamine in a suitable solvent or mixture of solvents such as dioxane and water, or dichloromethane to yield **XXVII** as described by Beeli, R.; Steger, M.; Linden, A.; Robinson, J. A. *Helv. Chim. Acta* **1996**, *79*, 2235-2248.

Reaction Scheme 7



XXVIII ——————→ XXIX

i: **XXVIII** can be synthesized according to P. Waldmeier, "Solid-supported synthesis of highly substituted xanthene-derived templates for the synthesis of β-turn stabilized cyclic peptide libraries", PhD-thesis, University of Zurich, 1996. For cleaving the phthalimide group **XXVIII** is conveniently submitted to hydrazinolysis, e.g. by treatment with hydrazine hydrate in a suitable solvent such as ethanol at an elevated temperature, e.g. at about 80° C.

ii: The intermediate aminonitrile is saponified, conveniently under basic conditions, e.g. with aqueous sodium hydroxide in a suitable solvent such as ethanol at an elevated temperature, conveniently under reflux, to yield **XXIX**.

XXIX ——————→ XXX

iii: The intermediate free amino acid formed is conveniently protected with reagents such as 9-fluorenylmethoxcarbonyl chloride or 9-fluorenylmethoxcarbonyl succinimide using a base such as sodium carbonate or triethylamine in a suitable solvent or mixture of solvents such as dioxane and water, or dichloromethane to yield **XXX** as described by P. Waldmeier, "Solid-supported synthesis of highly substituted xanthene-derived templates for the synthesis of β-turn stabilized cyclic peptide libraries", PhD-thesis, University of Zurich, 1996.

XXIX ——————→ XXXI

iv: Regioselective bromination of **XXIX** is performed preferably with bromine in acetic acid and dichloromethane. In a similar fashion $R^6 = NO_2$ can be introduced by treatment with HNO_3 in acetic acid and $R^6 = CH_2$-NPt by treatment with hydroxymethyl phthalimide in H_2SO_4.

v: The amino group is conveniently Z-protected with reagents such as benzyloxycarbonyl chloride or succinimide in a suitable solvent such as dioxane in presence of a base such as aqueous sodium hydroxide.

vi: The carboxylic acid group is esterified, preferably with DBU and methyl iodide in DMF.

XXXI ——————→ XXXII

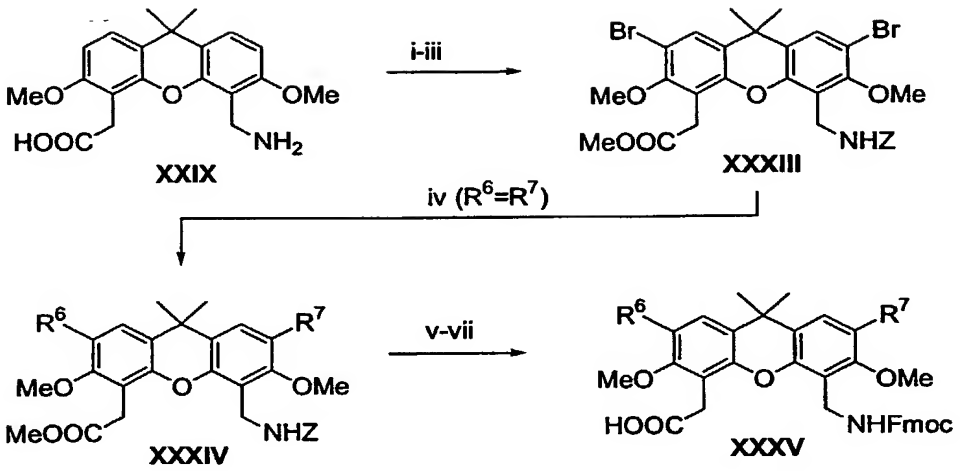
vii: Introduction of lower alkyl, substituted lower alkyl and aryl substituents (R^6), conveniently by Palladium(0)- catalyzed Stille- (Stille, J.K. *Angew. Chem.*1986, *68*, 504) and Suzuki- couplings (Oh-e, T.; Mijaura, N.; Suzuki, A. *J. Org. Chem.* **1993**, *58*, 2201).

viii: Removal of the Z-group, e.g. by hydrogenation using H_2 and a catalyst such as Palladium on charcoal in a suitable solvent such as ethanol, DMF and ethyl acetate.

ix: Hydrolysis of the ester group, conveniently under acidic conditions, e.g. with 25% aqueous hydrochloric acid in a suitable solvent such as dioxane at an elevated temperature, preferably at about 100° C.

x: The intermediate free amino acid formed is conveniently protected with reagents such as 9-fluorenylmethoxcarbonyl chloride or 9-fluorenylmethoxcarbonyl succinimide using a base such as sodium carbonate or triethylamine in a suitable solvent or mixture of solvents such as dioxane and water, or dichloromethane to yield **XXXII**.

Reaction Scheme 8



XXIX ⟶ XXXIII

i: Double ortho- bromination is performed preferably with excess bromine in acetic acid and dichloromethane. In a similar fashion $R^6 = R^7 = NO_2$ can be introduced by treatment with HNO_3 in acetic acid and $R^6 = R^7 = CH_2$-NPt by treatment with hydroxymethyl phthalimide in H_2SO_4.

ii: The amino group is protected, conveniently Z-protected, with reagents such as benzyloxycarbonyl chloride or succinimide in a suitable solvent such as dioxane in the presence of a base such as aqueous sodium hydroxide.

iii: The carboxylic acid group is esterified, preferably with DBU and methyl iodide in DMF to yield **XXXIII**.

XXXIII ⟶ XXXIV

iv: Introduction of lower alkyl, substituted lower alkyl and aryl substituents ($R^6 = R^7$), e.g. by Palladium(0)- catalyzed Stille- (Stille, J.K. *Angew. Chem.***1986**, *68*, 504) and Suzuki-couplings (Oh-e, T.; Mijaura, N.; Suzuki, A. *J. Org. Chem.* **1993**, *58*, 2201).

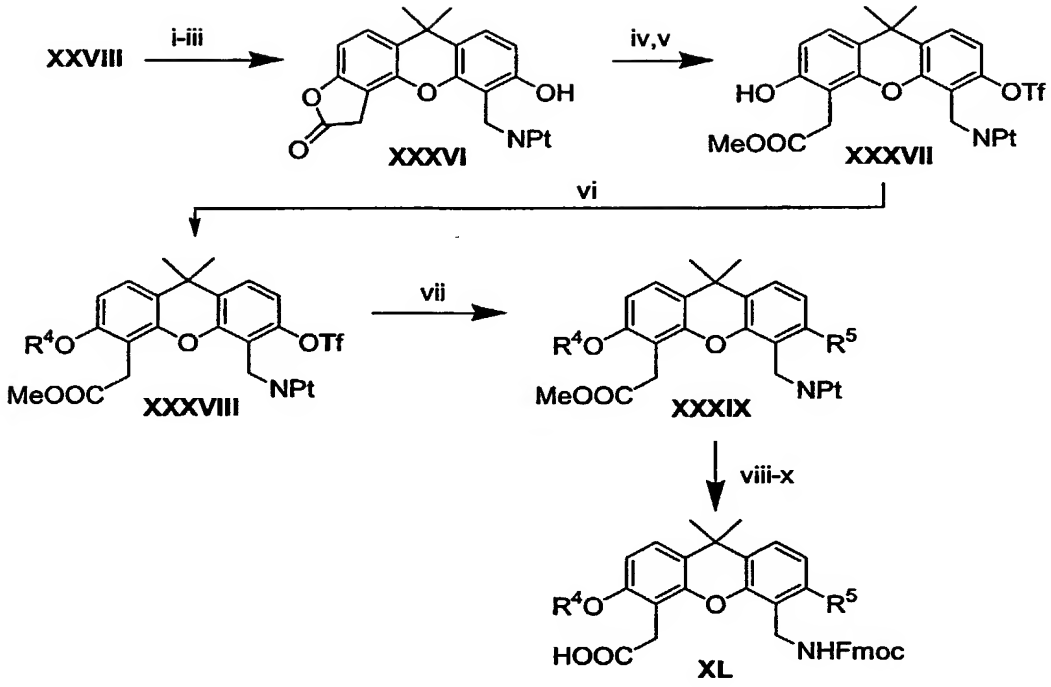
XXXIV ⟶ XXXV

v: Removal of the Z-group of **XXXIV**, e.g. by hydrogenation using H_2 and a catalyst such as Palladium on charcoal in a suitable solvent such as ethanol, DMF or ethyl acetate.

vi: Hydrolysis of the ester group, conveniently under acidic conditions, e.g. with 25% aqueous hydrochloric acid in a suitable solvent such as dioxane at an elevated temperature, conveniently at about 100° C.

vii: The intermediate free amino acid formed is conveniently protected with reagents such as 9-fluorenylmethoxcarbonyl chloride or 9-fluorenylmethoxcarbonyl succinimide using a base such as sodium carbonate or triethylamine in a suitable solvent or mixture of solvents such as dioxane and water, or dichloromethane to yield **XXXV**.

Reaction Scheme 9



XXVIII ⟶ XXXVI

i: Cleavage of the methoxy groups of **XXVIII**, preferably by treatment with an excess of boron tribromide in a suitable solvent such as dichloromethane.

ii: Hydrolysis of the cyano group under acidic conditions, preferably with 25% aqueous hydrochloric acid in a suitable solvent such as dioxane at an elevated temperature, conveniently at about 100° C.

iii: The resulting acid is treated with a dehydrating agent such as thionyl chloride in a suitable solvent such as dioxane yields **XXXVI**.

XXXVI ——————————→ XXXVII

iv: Treatment of **XXXVI** with an appropriate triflating reagent, preferably trifluorosulfonic acid anhydride in the presence of a base such as 2,6-di-tert.-butyl-pyridine in a suitable solvent such as dichloromethane.

v: Heating of the intermediate, conveniently in a suitable solvent such as methanol.

XXXVII ——————————→ XXXVIII

vi: Introduction of lower alkyl or aryl-lower alkyl (R^4) by alkylation.

XXXVIII ——————————→ XXXIX

vii: Introduction of lower alkyl or aryl (R^5), conveniently by Palladium(0)- catalyzed Suzuki-coupling (Oh-e, T.; Mijaura, N.; Suzuki, A. *J. Org. Chem.* **1993**, *58*, 2201).

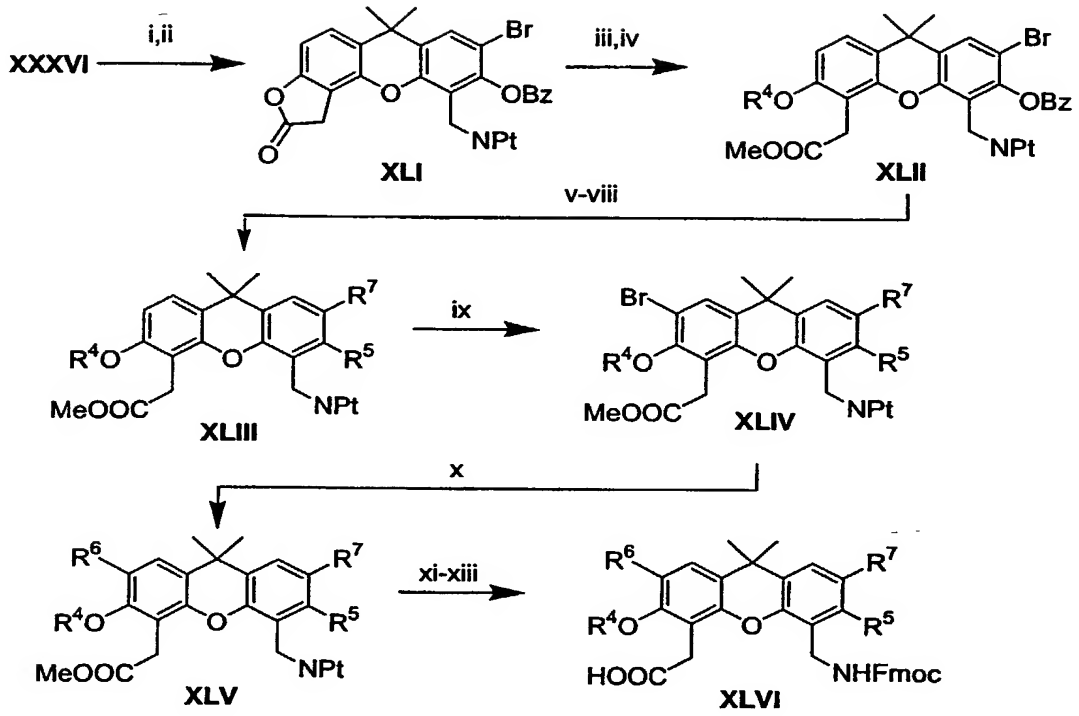
XXXIX ——————————→ XL

viii: Hydrolysis of the ester group under acidic conditions, conveniently with 25% aqueous hydrochloric acid in a suitable solvent such as dioxane at an elevated temperature, e.g. at about 100° C.

ix: Cleavage of the phthalimido group, conveniently by hydrazinolysis, e.g. with hydrazine hydrate in a suitable solvent such as ethanol.

x: The intermediate free amino acid formed is conveniently protected with reagents such as 9-fluorenylmethoxcarbonyl chloride or 9-fluorenylmethoxcarbonyl succinimide using a base such as sodium carbonate or triethylamine in a suitable solvent or mixture of solvents such as dioxane and water, or dichloromethane to yield **XL**.

Reaction Scheme 10



XXXVI ——————→ XLI

i: Bromination of **XXXVI** using reagents such as bromine in a mixture of acetic acid and dichloromethane at temperatures ranging from about 0° C to about room temperature.

ii: Benzoylation of the hydroxy group using an appropriate acylating agent such as benzoyl chloride or benzoic acid anhydride, a base such as pyridine or triethylamine and a suitable solvent such as dichloromethane.

XLI ——————→ XLII

iii: **XLI** is treated with methanol and a catalytic amount of an acidic catalyst such as campher sulfonic acid under heating.

iv: Introduction of lower alkyl or aryl-lower alkyl (R^4) by alkylation using a base such as sodium hydride or potassium tert.-butoxide in a solvent such as tetrahydrofuran, dimethoxyethane or DMF.

XLII ⟶ XLIII

v: Lower alkyl, substituted lower alkyl and aryl substituents (R^7) are introduced, e.g. by Palladium(0)- catalyzed Stille- (Stille, J.K. *Angew. Chem.***1986**, *68*, 504) and Suzuki-couplings (Oh-e, T.; Mijaura, N.; Suzuki, A. *J. Org. Chem.* **1993**, *58*, 2201).

vi: For cleaving the benzyloxy group the intermediate is conveniently heated with sodium cyanide adsorbed on aluminum oxide and methanol.

vii: Treatment with an appropriate triflating reagent, preferably trifluorosulfonic acid anhydride, in the presence of a base such as 2,6-di-tert.-butyl-pyridine in a suitable solvent such as dichloromethane.

viii: Introduction of lower alkyl and aryl substituents (R^5), e.g. by Palladium(0)- catalyzed Stille- (Stille, J.K. *Angew. Chem.***1986**, *68*, 504) and Suzuki- couplings (Oh-e, T.; Mijaura, N.; Suzuki, A. *J. Org. Chem.* **1993**, *58*, 2201).

XLIII ⟶ XLIV

ix: Bromination under standard conditions such as using bromine in acetic acid and dichloromethane at temperatures ranging from about 0° C to about room temperature.

XLIV ⟶ XLV

x: Lower alkyl, substituted lower alkyl and aryl substituents (R^6) are introduced, e.g. by Palladium(0)- catalyzed Stille- (Stille, J.K. *Angew. Chem.***1986**, *68*, 504) and Suzuki-couplings (Oh-e, T.; Mijaura, N.; Suzuki, A. *J. Org. Chem.* **1993**, *58*, 2201).

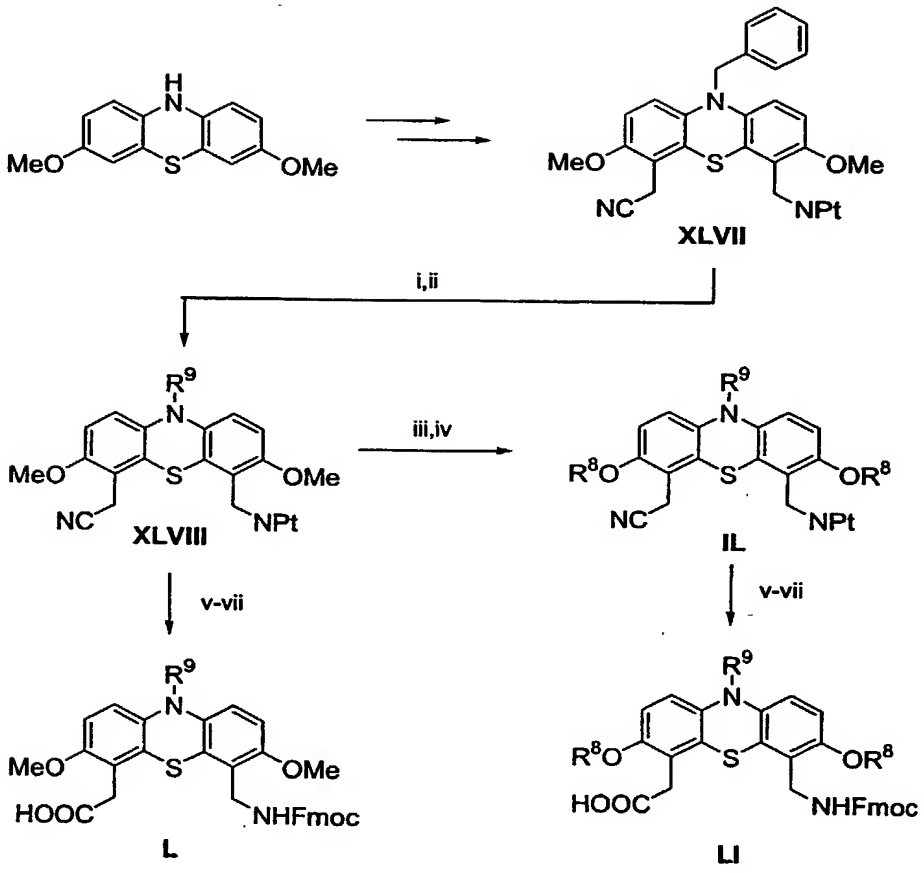
XLV ⟶ XLVI

xi: The ester group is hydrolyzed under acidic conditions, conveniently with 25% aqueous hydrochloric acid in a suitable solvent such as dioxane at an elevated temperature, e.g. at about 100° C.

xii: The phthalimido group is cleaved, e.g. by hydrazinolysis, conveniently with hydrazine hydrate in a suitable solvent such as ethanol.

xiii: The intermediate free amino acid formed is conveniently protected with reagents such as 9-fluorenylmethoxcarbonyl chloride or 9-fluorenylmethoxcarbonyl succinimide using a base such as sodium carbonate or triethylamine in a suitable solvent or mixture of solvents such as dioxane and water, or dichloromethane to yield **XLVI**.

Reaction Scheme 11



XLVII ⟶ XLVIII

i: 3,7-Dimethoxyphenothiazine is prepared and converted into **XLVII** according to Müller, K.; Obrecht, D.; Knierzinger, A.; Spiegler, C.; Bannwarth, W.; Trzeciak, A.; Englert, G.; Labhardt, A.; Schönholzer, P. *Perspectives in Medicinal Chemistry*, Editor Testa, B.; Kyburz, E.; Fuhrer, W.; Giger, R., Weinheim, New York, Basel, Cambridge: Verlag Helvetica Chimica Acta, **1993,** 513-531; Bannwarth, W.; Gerber, F.; Grieder, A.; Knierzinger, A.; Müller, K.; Obrecht. D.; Trzeciak, A. *Can. Pat. Appl.* CA2101599(131 pages). The benzyl group is cleaved off from **XLVII** conveniently by hydrogenation, e.g.

with H_2 and a catalyst such as Palladium on charcoal in a suitable solvent such as ethanol, DMF or ethyl acetate.

ii: Introduction of lower alkyl (R^9) by alkylation using an appropriate alkylating agent (R^9-X'; X'.= OTf, Br, I) and strong bases such as sodium amide in liquid nitrogen or sodium hydride in tetrahydrofuran, dioxan or DMF in the presence of a phase transfer catalyst such as TDA-I. In a similar manner substituted lower alkyl (R^9) can be introduced; thus, for example $R^9 = CH_2COOR^{10}$ and $CH_2CH_2COOR^{10}$ can be introduced by treatment with the appropriate 2-halo acetic and, respectively, 3-halo propionic acid derivatives.

XLVIII ⟶ **IL**

iii: Cleavage of the methoxy groups of **XLVIII**, conveniently by treatment with an excess of boron tribromide in a suitable solvent such as dichloromethane at temperatures ranging from about –20° C to about room temperature.

iv: For the introduction of lower alkyl, substituted lower alkyl or aryl-lower alkyl substituents (R^8) the intermediate bis-phenol derivative is conveniently reacted with a reagent of the formula R^8-X' (X' = OTf, Br, I) in the presence of strong bases such as sodium hydride in tetrahydrofuran, dioxan or DMF in the presence of a phase transfer catalyst such as TDA-I.

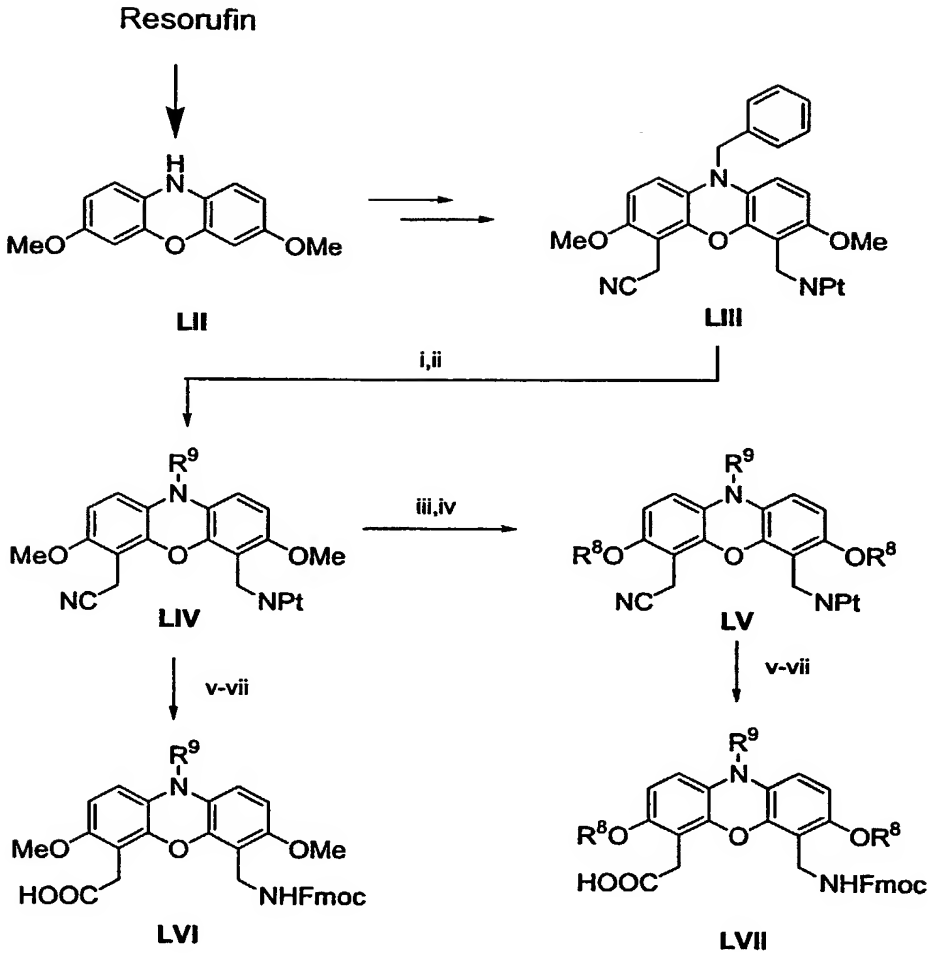
XLVIII ⟶ **L** **IL** ⟶ **LI**

v: The cyano group of **XLVIII** and, respectively, **IL** is hydrolyzed, conveniently under acidic conditions, e.g. with 25% aqueous hydrochloric acid in a suitable solvent such as dioxane at an elevated temperature, e.g. at about 100° C.

vi: The phthalimide group of the intermediate is cleaved, conveniently by hydrazinolysis, e.g. with hydrazine hydrate in a suitable solvent such as ethanol.

vii: The free amino group is conveniently protected with reagents such as 9-fluorenylmethoxcarbonyl chloride or 9-fluorenylmethoxcarbonyl succinimide using a base such as sodium carbonate or triethylamine in a suitable solvent or mixture of solvents such as dioxane and water, or dichloromethane to yield **L** and, respectively, **LI**.

Reaction Scheme 12



LIII ⟶ LIV

i: **LII** can be prepared from commercial resorufin and coverted into **LIII** according to Müller, K.; Obrecht, D.; Knierzinger, A.; Spiegler, C.; Bannwarth, W.; Trzeciak, A.; Englert, G.; Labhardt, A.; Schönholzer, P. *Perspectives in Medicinal Chemistry*, Editor Testa, B.; Kyburz, E.; Fuhrer, W.; Giger, R., Weinheim, New York, Basel, Cambridge: Verlag Helvetica Chimica Acta, **1993**, 513-531; Bannwarth, W.; Gerber, F.; Grieder, A.; Knierzinger, A.; Müller, K.; Obrecht. D.; Trzeciak, A. *Can. Pat. Appl.* CA2101599(131 pages). For splitting off the benzyl group **LIII** is conveniently hydrogenated e.g. with H_2

and a catalyst such as Palladium on charcoal in a suitable solvent such as ethanol, DMF or ethyl acetate.

ii: Introduction of lower alkyl (R^9) by alkylation with R^9-X' (X' = OTf, Br, I) using strong bases such as sodium amide in liquid nitrogen or sodium hydride in tetrahydrofuran, dioxan or DMF in the presence of a phase transfer catalyst such as TDA-I to yield **LIV**. In a similar manner substituted lower alkyl (R^9) can be introduced; thus, for example, R^9 = CH_2COOR^{10} and $CH_2CH_2COOR^{10}$ can be introduced by treatment with the appropriate 2-halo acetic and, respectively, 3-halo propionic acid derivatives.

LIV ⟶ **LV**

iii: Cleavage of the methoxy groups of **LIV**, conveniently by treatment with excess boron tribromide in dichloromethane at temperatures ranging from about –20° to about room temperature.

iv: The intermediate bis-phenol derivative is preferably reacted with R^8-X' (X'= OTf, Br, I) in the presence of strong bases such as sodium hydride in tetrahydrofuran, dioxan or DMF in the presence of a phase transfer catalyst such as TDA-I.

LIV ⟶ **LVI** **LV** ⟶ **LVII**

v: The cyano group of **LIV** and, respectively, **LV** is hydrolyzed under acidic conditions, e.g. with 25% aqueous hydrochloric acid in a suitable solvent such as dioxane at an elevated temperature, conveniently at about 100° C.

vi: The phthalimide group is cleaved, conveniently by hydrazinolysis, e.g. with hydrazine hydrate in suitable solvent such as ethanol.

vii: The free amino group is conveniently protected with reagents such as 9-fluorenylmethoxcarbonyl chloride or 9-fluorenylmethoxcarbonyl succinimide using a base such as sodium carbonate or triethylamine in suitable solvent or mixture of solvents such as dioxane and water, or dichloromethane to yield **LVI** and, respectively, **LVII.**

The following Examples illustrate the invention in more detail but are not intended to limit its scope in any manner.

Example 1

Preparation of a single compound of formula I

1,4 g of 2-chlorotrityl chloride resin (1.25 mmol/g, 1.75 mmol) were filled into a three necked flask. The resin was suspended in DCM (14 ml) and allowed to swell at room temperature under constant stirring. The resin was treated with 1.25 g (1.077 equiv.) of Fmoc-Arg(Pmc)-OH and 0.898 ml of diisopropylethylamine (DIPEA) in DCM (10 ml), the mixture was shaken at 25°C for 15 minutes, poured into the pre-swollen resin and stirred at 25°C for 18 hours. The resin colour changed to purple and the solution remained yellowish. The resin was washed extensively and dried at 40°C under vacuum for 4 hours.

Yield: 2.379 gm Loading: 84 %

The esterified resin was then subjected to the following synthesis cycle → 40 mg per reaction vessel .

Step	Reagent	Time
1	DCM, swell and wash	3 x 1 min.
2	20 % piperidine/DMF	1 x 15 min.
3	DMF, wash and swell	5 x 1 min.
4	4 equiv. Fmoc amino acid/DMF + 4 equiv. 1-benzotriazol-1-yl-tetramethylurounium hexafluoro phosphate (HBTU) + 4 equiv. 1-hydroxybenzotriazole (HOBt) + 6 equiv. Diisopropylethylamine	1 x 120 min.
5	DMF, wash	3 x 1 min.
6	Isopropylalcohol, wash	2 x 1 min.

7	DCM, wash	2 x 1 min.

5 ml of the solvent were used in each step. Fmoc-Val-OH, Fmoc-Ile-OH, Fmoc-Glu(OtBu)-OH, Fmoc-L-Pro-OH, Fmoc-D-Pro-OH, Fmoc-Ile-OH, Fmoc-Pro-OH, Fmoc-Lys(Boc)-OH and Fmoc-Lys(Boc)-OH were coupled according to the above protocol.

Cleavage of the fully protected peptide fragment

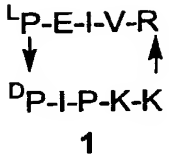
After completion of the synthesis, the peptide resin was suspended in 5 ml of 1 % TFA in DCM (v/v) and agitated for 10 minutes, whereupon the resin was filtered off and the filtrate was neutralized with pyridine (1 equiv.). This procedure was repeated twice to ensure completion of the cleavage. The filtrate was evaporated to dryness and analyzed by reverse phase (RP)-HPLC to monitor the efficiency of the linear peptide synthesis.

Cyclization of the H-Lys(Boc)-Lys(Boc)-Pro-Ile-D-Pro-L-Pro-Glu(OtBu)-Ile-Val-Arg(Pmc)-OH linear peptide [SEQ ID NO:31]

50 mg(0.0294 mmol) of the fully protected linear peptide were dissolved in DMF (50 ml, conc. 1 mg/ml). Then 33.5 mg (0.0882mMol), 3 equiv.) of HATU, 12.0mg (0.0882mMol), 3 eq) of HOAt and 5 ml of DIPEA (1% v/v) were added and the mixture was stirred at 20°C for 16 hours and subsequently concentrated in a vacuum. The residue was partitioned between dichloromethane (DCM) and H_2O/CH_3CN (90 : 10). The DCM phase was evaporated to yield the pure fully protected cyclic peptide.

Deprotection of the cyclic peptide:

The amorphous powder obtained was dissolved in 2 ml of the cleavage mixture containing 95% trifluoroacetic acid, 2.5% water and 2.5% triisopropyl siliane (TIS). The mixture was left to stand at 20°C for 2 hours and then concentrated in a vacuum. The residue was triturated with diethyl ether, and 20 mg of compound **1** [SEQ ID NO:32] were obtained as a white colored powder.



1

$C_{55}H_{95}N_{15}O_{12}$, MW 1158.5

MS(ESI): 580.02 $(M+2H^+)^{2+}$, 387.02 $(M+3H^+)^{3+}$

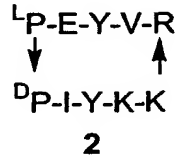
HPLC-RT(min.): 7.51

Conditions: Analytical HPLC-conditions: column CC 250/4 Nucleosil 100-5 Protect 1 (Ser. No. 8111346, Batch 8023); gradient: 5 % acetonitril / water(0.1% trifluoroacetic acid to 100% acetonitril in 15 minutes; stay constant for 5 minutes and return to 5 % acetonitril / water(0.1% trifluoroacetic acid) in 5 minutes.

Example 2

Preparation of a single compound of formula I

By a procedure analogous to that described in Example 1, Fmoc-Val-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Glu(OtBu)-OH, Fmoc-^{L}Pro-OH, Fmoc-^{D}Pro-OH, Fmoc-Ile-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Lys(Boc)-OH and Fmoc-Lys(Boc)-OH were coupled to the Fmoc-Arg(Pmc)-OH loaded resin, cleaved, cyclised and deprotected to yield compound **2** [SEQ ID NO:33]:



2

$C_{62}H_{95}N_{15}O_{14}$, MW 1274.5

MS(ESI): 638.4 $(M+2H^+)^{2+}$, 424.8.02 $(M+3H^+)^{3+}$

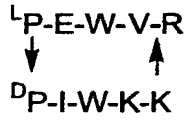
HPLC-RT(min.): 8.59

Conditions: Analytical HPLC-conditions: column CC 250/4 Nucleosil 100-5 Protect 1 (Ser. No. 8111346, Batch 8023); gradient: 5 % acetonitril / water(0.1% trifluoroacetic acid to 100% acetonitril in 15 minutes; stay constant for 5 minutes and return to 5 % acetonitril / water(0.1% trifluoroacetic acid) in 5 minutes.

Example 3

Preparation of a single compound of formula I

By a procedure analogous to that described in Example 1, Fmoc-Val-OH, Fmoc-Trp-OH, Fmoc-Glu(OtBu)-OH, Fmoc-^{L}Pro-OH, Fmoc-^{D}Pro-OH, Fmoc-Ile-OH, Fmoc-Trp-OH, Fmoc-Lys(Boc)-OH and Fmoc-Lys(Boc)-OH were coupled to the Fmoc-Arg(Pmc)-OH loaded resin, cleaved, cyclised and deprotected to yield compound **3** [SEQ ID NO:34]:



3

$C_{66}H_{97}N_{17}O_{12}$, MW 1320.6

MS(ESI): 1321.6 $(M+H^{+})^{+}$

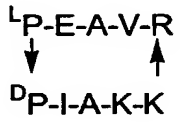
HPLC-RT(min.): 9.04

Conditions: Analytical HPLC-conditions: column CC 250/4 Nucleosil 100-5 Protect 1 (Ser. No 8111346, Batch 8023); gradient: 5 % acetonitril / water(0.1% trifluoroacetic acid to 100% acetonitril in 15 minutes; stay constant for 5 minutes and return to 5 % acetonitril / water(0.1% trifluoroacetic acid) in 5 minutes.

Example 4

Preparation of a single compound of formula I

By a procedure analogous to that described in Example 1, Fmoc-Val-OH, Fmoc-Ala-OH, Fmoc-Glu(OtBu)-OH, Fmoc-^{L}Pro-OH, Fmoc-^{D}Pro-OH, Fmoc-Ile-OH, Fmoc-Ala-OH, Fmoc-Lys(Boc)-OH and Fmoc-Lys(Boc)-OH were coupled to the Fmoc-Arg(Pmc)-OH loaded resin, cleaved, cyclised and deprotected to yield compound **4** [SEQ ID NO:35]:



4

$C_{50}H_{87}N_{15}O_{12}$, MW 1090.5

MS(ESI): 546.15.4 $(M+2H^{+})^{2+}$, 364.3 $(M+3H^{+})^{3+}$

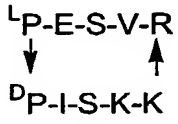
HPLC-RT(min.): 12.51

Conditions: Analytical HPLC-conditions: column CC 250/4 Nucleosil 100-5 Protect 1 (Ser. No. 8111346, Batch 8023); gradient: 5 % acetonitril / water(0.1% trifluoroacetic acid to 100% acetonitril in 15 minutes; stay constant for 5 minutes and return to 5 % acetonitril / water(0.1% trifluoroacetic acid) in 5 minutes.

Example 5

Preparation of a single compound of formula I

By a procedure analogous to that described in Example 1, Fmoc-Val-OH, Fmoc-Ser(OtBu)-OH, Fmoc-Glu(OtBu)-OH, Fmoc-^{L}Pro-OH, Fmoc-^{D}Pro-OH, Fmoc-Ile-OH, Fmoc-Ser(OtBu)-OH, Fmoc-Lys(Boc)-OH and Fmoc-Lys(Boc)-OH were coupled to the Fmoc-Arg(Pmc)-OH loaded resin, cleaved, cyclised and deprotected to yield compound **5** [SEQ ID NO:36]:



5

$C_{50}H_{87}N_{15}O_{14}$, MW 1122.3

MS(ESI): 562.15 $(M+2H^+)^{2+}$, 375.3 $(M+3H^+)^{3+}$

HPLC-RT(min.): 5.74

Conditions: Analytical HPLC-conditions: column CC 250/4 Nucleosil 100-5 Protect 1 (Ser. No. 8111346, Batch 8023); gradient: 5 % acetonitril / water(0.1% trifluoroacetic acid to 100% acetonitril in 15 minutes; stay constant for 5 minutes and return to 5 % acetonitril / water(0.1% trifluoroacetic acid) in 5 minutes.

Example 6

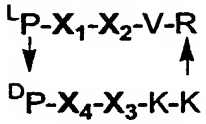
Synthesis of a library of compounds of formula I for mimicking the PDGF-loop-III on a diproline template and testing thereof in a solid-phase assay

1.Target peptides

x_1-x_4: variable amino acid residues (x)

ValArgLysLys (VRKK) [SEQ ID NO:1]: constant amino acid residues

^{D}Pro^{L}Pro: template



[SEQ ID NO:37]

x_1^{1-4}: Glu, Tyr, Trp, Ala

x_2^{1-6}: Ile, Tyr, Trp, Ala, Ser, Lys

x_3^{1-6}: Pro, Tyr, Trp, Ala, Ser, Lys

x_4^{1-4}: Ile, Tyr, Trp, Ala

Table 1. The sequences of the 24 target cyclic peptides (the first five corresponding to those obtained according to Examples 1-5)

	$x_1^1x_4^1$ (E-I)	$x_1^2x_4^2$ (Y-Y)	$x_1^3x_4^3$ (W-W)	$x_1^4x_4^4$ (A-A)
$x_2^1x_3^1$ (I-P)	**1** ^{L}P-E-I-V-R ^{D}P-I-P-K-K [SEQ ID NO:32]	**7** ^{L}P-Y- I-V-R ^{D}P-Y-P-K-K [SEQ ID NO:39]	**13** ^{L}P-W- I-V-R ^{D}P-W-P-K-K [SEQ ID NO:45]	**19** ^{L}P-A- I-V-R ^{D}P-A-P-K-K [SEQ ID NO:51]
$x_2^2x_3^2$ (Y-Y)	**2** ^{L}P-E-Y-V-R ^{D}P- I-Y-K-K [SEQ ID NO:33]	**8** ^{L}P-Y-Y-V-R ^{D}P-Y-Y-K-K [SEQ ID NO:40]	**14** ^{L}P-W-Y-V-R ^{D}P-W-Y-K-K [SEQ ID NO:46]	**20** ^{L}P-A-Y-V-R ^{D}P-A-Y-K-K [SEQ ID NO:52]
$x_2^3x_3^3$ (W-W)	**3** ^{L}P-E-W-V-R ^{D}P-I-W-K-K [SEQ ID NO:34]	**9** ^{L}P-Y-W-V-R ^{D}P-Y-W-K-K [SEQ ID NO:41]	**15** ^{L}P-W-W-V-R ^{D}P-W-W-K-K [SEQ ID NO:47]	**21** ^{L}P-A-W-V-R ^{D}P-A-W-K-K [SEQ ID NO:53]
$x_2^4x_3^4$ (A-A)	**4** ^{L}P-E-A-V-R ^{D}P- I-A-K-K [SEQ ID NO:35]	**10** ^{L}P-Y-A-V-R ^{D}P-Y-A-K-K [SEQ ID NO:42]	**16** ^{L}P-W-A-V-R ^{D}P-W-A-K-K [SEQ ID NO:48]	**22** L-P-A-A-V-R D-P-A-A-K-K [SEQ ID NO:54]

$x_2^5x_3^5$ (S-S)	**5** ^{L}P-E-S-V-R ^{D}P-I-S-K-K [SEQ ID NO:36]	**11** ^{L}P-Y-S-V-R ^{D}P-Y-S-K-K [SEQ ID NO:43]	**17** ^{L}P-W-S-V-R ^{D}P-W-S-K-K [SEQ ID NO:49]	**23** ^{L}P-A-S-V-R ^{D}P-A-S-K-K [SEQ ID NO:55]
$x_2^6x_3^6$ (K-K)	**6** ^{L}P-E-K-V-R ^{D}P-I-K-K-K [SEQ ID NO:38]	**12** ^{L}P-Y-K-V-R ^{D}P-Y-K-K-K [SEQ ID NO:44]	**18** ^{L}P-W-K-V-R ^{D}P-W-K-K-K [SEQ ID NO:50]	**24** ^{L}P-A-K-V-R ^{D}P-A-K-K-K [SEQ ID NO:56]

2. Experimental procedures:

2.1. Synthesis of protected linear peptides

The first amino acid Fmoc-Arg(Pmc)-OH (1 eq.) was linked to 2-chlorotrityl chloride resin (Polyphor, 1.25mmol/g) with 3 eq. DIEA in DCM overnight, the attachment was ca.85%. The linear peptides were assembled using standard Fmoc chemistry, 4 eq. each of amino acids, of HBTU and HOBt and 6 eq. of DIEA in DMF being used and the coupling time being 1.5-2 h. The protected linear peptides were cleaved from the resin with 1% TFA in DCM (2× 10 min.) and neutralized with pyridine (1 eq.), then the solvent was evaporated.

2.2. Cyclisation of protected linear peptides

The protected linear peptide (without purification) was directly cyclized at a concentration of 1.0 mg/ml in DMF using HATU (3 eq.), HOAt (3 eq.) and DIEA (1% v/v) for 16 h. Then DMF and DIEA were evaporated, the residue was dissolved in DCM, the solution was extracted with H_2O/CH_3CN (90:10), and afterwards the DCM was removed.

2.3. Deprotection of the cyclized peptides

The cyclization product was treated with 95% TFA, 2.5% H_2O and 2.5% TIS for 2 h, then most of the TFA was evaporated. Et_2O was added to precipitate the product. After centrifugation, the ether was carefully removed and the final product was obtained after drying under reduced pressure. Depending on its purity, the product was purified by preparative HPLC.

2.4. Solid-phase assay

Direct immobilization of platelet-derived growth factor β- receptor (PDGFR-β) was performed by overnight incubation in immunosorbent 96-well plates (Nunc) at 4°C using 100ng of purified protein in 100µl of 15mM Na_2CO_3, 35mM $NaHCO_3$, pH 9.6. Plates were washed once with Tris-buffered saline (TBS, 20mM Tris-HCl, 150mM Na Cl, pH 7.4), and nonspecific adsorption was blocked by at least 1h of incubation with TBS plus 1% bovine serum albumin (BSA). Following washing with TBS plus 0.1% Tween, 3000cpm of ^{125}I-PDGF-BB and increasing amounts of unlabeled PDGF-BB or the peptides to be tested were added to duplicate wells and incubated for 3 h at room temperature in TBS plus 0.1% Tween, 1mM $CaCl_2$, 1mM $MgCl_2$, and 1% BSA. The plates were washed three times with TBS plus 0.1% Tween, and the bound ligand was removed with 0.1 M citric acid, pH 2.5, before counting in a γ-counter.

3. Results

The cyclic peptides were analyzed and purified by preparative HPLC (dual-pump *Pharmacia* system with *Waters RCM-µBondapak™-C_{18}*-cartridges, 10µm 300A 25×100mm for prep. and 8×100mm for anal., with flow rates of 8 and 2ml/mim, respectively; UV detection at 226 and 278nm), then MS, NMR(600MHz,1H) and CD. Solid-phase assays were run, as described in 2.4.

4. Discussion

4.1. Linear peptides were analyzed by HPLC, all of the 24 compounds turned out to be pure, >95% indicating that the assembling of amino acids worked performed reliably.

4.2 Cyclized peptides

a) The linear peptides cleaved from resin, neutralized with pyridine to form pyridine salts, which needed not to be purified before their cyclization.

b) Different concentrations of peptides for cyclization were compared, 1mg, 2mg, 5mg, 10mg, 20mg/ml DMF, the 1mg/ml concentration gave the best result.

c) The purities of the crude products are shown in Table 2.

4.3. Solid-phase assay

The IC_{50}-values are shown in Table 2. The differences in IC_{50}-values between the crude and purified peptides were only marginal.

Table 2. Summary of Examples 1-24

Target peptide	Formula M.W.	ESI-MS $[M+H^+]^+$; $[M+2H^+]^{2+}$; $[M+3H^+]^{3+}$	Retention time of HPLC (min)	Purity of crude product	Assay I_{50} (μM)
1	$C_{55}H_{95}N_{15}O_{12}$ 1158.5	580.02; 387.02	7.51	95 %	2200
2	$C_{62}H_{95}N_{15}O_{14}$ 1274.5	1274.8; 638.01; 425.75	8.59	95 %	2000
3	$C_{66}H_{97}N_{17}O_{12}$ 1320.6	1320.81; 661.06; 441.11	9.04	80 %	1500
4	$C_{50}H_{87}N_{15}O_{12}$ 1090.3	1090.54; 545.83; 364.26	12.5	90%	>2500
5	$C_{50}H_{87}N_{15}O_{14}$ 1122.3	1122.71; 562.07; 375.05	5.74	95 %	2500
6	$C_{56}H_{101}N_{17}O_{12}$ 1204.5	1205.7; 603.18; 402.58	9.90	95 %	> 2500
7	$C_{62}H_{95}N_{15}O_{12}$ 1242.5	621.90; 414.89	9.14	95 %	1500
8	$C_{69}H_{95}N_{15}O_{14}$ 1358.6	679.82; 453.61	9.42	95 %	800
9	$C_{73}H_{97}N_{17}O_{12}$ 1404.7	1404.83; 703.08; 469.16	9.71	65%	500
10	$C_{57}H_{87}N_{15}O_{12}$ 1174.4	1174.73; 587.97; 392.39	9.09	90 %	2000
11	$C_{57}H_{87}N_{15}O_{14}$ 1206.4	1206.75; 604.02; 403.01	9.10	90%	2000
12	$C_{63}H_{101}N_{17}O_{12}$ 1288.6	1288.92; 645.06; 430.47	8.59	90 %	1500
13	$C_{66}H_{97}N_{17}O_{10}$ 1288.6	1288.82; 645.08; 430.47	8.27	95 %	260

14	$C_{73}H_{97}N_{17}O_{12}$ 1404.7	1405.0; 703.09; 469.08	9.26	90 %	170
15	$C_{77}H_{99}N_{19}O_{10}$ 1450.8	1451.06; 726.06; 484.42	10.35	20 %	
16	$C_{61}H_{89}N_{17}O_{10}$ 1220.5	1220.87; 611.03; 407.74	9.81	90 %	800
17	$C_{61}H_{89}N_{17}O_{12}$ 1252.5	1252.85; 627.03; 418.46	9.84	90 %	800
18	$C_{67}H_{103}N_{19}O_{10}$ 1334.7	1334.78; 668.15; 445.80	9.10	90 %	500
19	$C_{50}H_{87}N_{15}O_{10}$ 1058.3	1058.84; 530.03; 353.69	7.86	95 %	>2500
20	$C_{57}H_{87}N_{15}O_{12}$ 1174.4	1174.71; 588.11; 392.41	8.20	60 %	2500
21	$C_{61}H_{89}N_{17}O_{10}$ 1220.5	1220.91; 611.16; 407.78	8.85	30 %	2000
22	$C_{45}H_{79}N_{15}O_{10}$ 990.2	495.7	6.77	85 %	>2500
23	$C_{45}H_{79}N_{15}O_{12}$ 1022.2	511.94	7.12	85 %	2500
24	$C_{51}H_{93}N_{17}O_{10}$ 1104.4	553.12	6.86	90 %	2500

Analytical HPLC-conditions: column CC 250/4 Nucleosil 100-5 Protect 1 (Ser. No. 8111346, Batch 8023); gradient: 5 % acetonitril / water(0.1% trifluoroacetic acid to 100% acetonitril in 15 minutes; stay constant for 5 minutes and return to 5 % acetonitril / water(0.1% trifluoroacetic acid) in 5 minutes.

Examples 25-40

The following Examples describe the application of the process to the synthesis of 6-mer, 8-mer, 10-mer, 12-mer, 14-mer and 16-mer β-hairpin loop mimetics incorporating three different templates and a common key motif **-k^1-x^1-template-x^2-k^2-** [SEQ ID NO:57] where x^1 = Y, F, K or W, x^2 = Y, k^1= K and k^2 = E. Due to the β-hairpin structure x^1 and x^2 are lying on the same side of the β-sheet and form a hydrophobic patch. Such motifs are present e.g. in various chemokines (see Tarby, C. M.; Saunders, J. *Drug Discovery Today* **1999**, *4*, 80-92; Ponath, P. D. *Exp. Opin. Invest. Drugs* **1998**, *7*, 1-16).

1. Synthesis of (2*S*,6*S*,8a*S*)-8a-{[(tert.-butyl)oxycarbonyl]methyl}perhydro-5,8-dioxo-{[(9H-fluoren-9-yl)methoxycarbonyl]amino}-pyrrolo[1,2-a]pyrazine-6-acetic acid (template **b1**):

To a stirred solution of 250mg (0.414mmol) of allyl {(2*S*,6*S*,8a*S*)-8a-[(tert.-butyl)oxycarbonyl] methyl}perhydro-5,8-dioxo-{[(9H-fluoren-9-yl)methoxycarbonyl]amino}-pyrrolo[1,2-a]pyrazin-6-acetate in a degassed mixture of dichloromethane/methanol (9:1, 3ml) were added under argon 25mg (0.0216mmol) of tetrakis(triphenylphosphine)palladium, 0.05ml of acetic acid and 0.025ml of N-methylmorpholin. The reaction mixture was stirred for 48 hours at room temperature and poured onto water and dichloromethane. The organic phase was dried ($MgSO_4$), evaporated and the residue chromatographed on SiO_2 with dichloromethane/methanol (9:1) to yield 180mg (77%) of (2*S*,6*S*,8a*S*)-8a-{[(tert.-butyl)oxycarbonyl]methyl}perhydro-5,8-dioxo-{[(9H-fluoren-9-yl)-methoxycarbonyl]amino}-pyrrolo[1,2-a]pyrazine-6-acetic acid (template **b1**) as a white powder.

^{1}H-NMR(300MHz, DMSO-d_6): 8.30 (s, 1H); 7.88 (d, J= 7.2, 2H); 7.67 (d, J=7.4, 2H); 7.62 (br.s, 1H); 7.41 (t, J= 7.2, 2H); 7.33 (t, J=7.4, 2H); 4.35-4.2 (m, 5H); 3.55 (br.d, J= 6.3, 2H); 2.8-2.55 (m, 3H); 2.45-2.25 (m, 2H); 2.1-1.95 (m, 1H); 1.35 (s, 9H); MS(ESI): 586.1 $(M+Na)^+$, 564.1 $(M+H)^+$.

2. Synthesis of linear peptides:

The first amino acid Fmoc-Arg(Pmc)-OH (1 eq.) was linked to 2-chlorotrityl chloride resin (Polyphor, 1.25mmol/g) with 3 eq. DIEA in DCM overnight, the attachment was ca.80%. The linear peptides were assembled using standard Fmoc chemistry, 4 eq. each of amino acids and of

the template (or, if appropriate, of Fmoc-^{L}Pro-OH and of Fmoc-^{D}Pro-OH), 4eq. each of HBTU and HOBt and 6 eq. of DIEA in DMF being used and the coupling time being 1.5-2 h. The protected linear peptides were cleaved from the resin with 1% TFA in DCM (4× 10 min.) and neutralized with pyridine (1 eq.), then the solvent was evaporated.

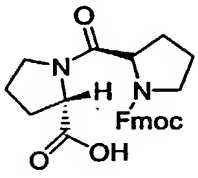
3. Cyclisation of the linear peptides

The protected linear peptide (without purification) was directly cyclized at a concentration of 1.0 mg/ml in DMF using HATU (3 eq.), HOAt (3 eq.) and DIEA (1% v/v) for 16 h. Then DMF and DIEA were evaporated, the residue was dissolved in DCM, the solution was extracted with H_2O/CH_3CN (90:10), and afterwards the DCM was removed.

4. Deprotection of the cyclized peptides

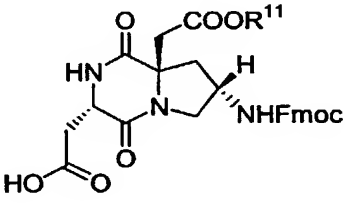
The cyclization product was treated with 95% TFA, 2.5% H_2O and 2.5% TIS for 2 h, then most of the TFA was evaporated. Et_2O was added to precipitate the product. After centrifugation, the ether was carefully removed and the final product was obtained after drying under reduced pressure. Depending on its purity, the product was purified by preparative HPLC.

The following templates were used:



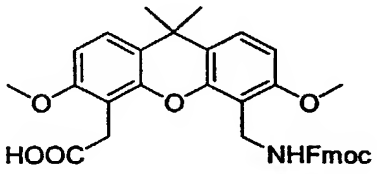
Template **a1**

(^{D}Pro-^{L}Pro)



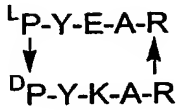
Template **b1** (R^{11}=tBu)

Template **b2** (R^{11}=H)



Template **f1**

Example 25



25

By a procedure analogous to that described in Example 1, Fmoc-Ala-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-^{L}Pro-OH, Fmoc-^{D}Pro-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ala-OH and Fmoc-Arg(Pmc)-OH were coupled to the Fmoc-Arg(Pmc)-OH loaded resin, cleaved, cyclised and deprotected to yield compound **25** [SEQ ID NO:58].

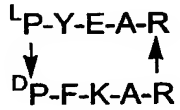
MW: $C_{57}H_{85}N_{17}O_{14}$, [1232.30]

MS(ESI): 616.72 $[M+2H^+]^{2+}$

HPLC-RT(min.): 10.83

Conditions: Analytical HPLC-conditions: column CC 250/4 Nucleosil 100-5 Protect 1 (Ser. No. 8111346, Batch 8023); gradient: 10 % acetonitril / 90 % water(containing 0.1% trifluoroacetic acid) to 100% acetonitril in 15 minutes; stay constant for 4 minutes and return to10 % acetonitril / water(0.1% trifluoroacetic acid) in 4 minutes.

Example 26:



26

By a procedure analogous to that described in Example 1, Fmoc-Ala-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-^{L}Pro-OH, Fmoc-^{D}Pro-OH, Fmoc-Phe-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ala-OH and Fmoc-Arg(Pmc)-OH were coupled to the Fmoc-Arg(Pmc)-OH loaded resin, cleaved, cyclised and deprotected to yield compound **26** [SEQ ID NO:59].

MW: $C_{57}H_{85}N_{17}O_{13}$, [1216.41]

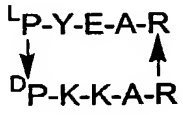
MS(ESI): 608.8 $[M+2H^+]^{2+}$

HPLC-RT(min.): 8.27

Conditions: Analytical HPLC-conditions: column CC 250/4 Nucleosil 100-5 Protect 1 (Ser. No. 8111346, Batch 8023); gradient: 10 % acetonitril/ 90 % water(containing 0.1% trifluoroacetic acid) to 100% acetonitril in 15 minutes; stay constant for

4 minutes and return to10 % acetonitril / water(0.1% trifluoroacetic acid) in 4 minutes.

Example 27:



27

By a procedure analogous to that described in Example 1, Fmoc-Ala-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-^{L}Pro-OH, Fmoc-^{D}Pro-OH, Fmoc-Lys(Boc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ala-OH and Fmoc-Arg(Pmc)-OH were coupled to the Fmoc-Arg(Pmc)-OH loaded resin, cleaved, cyclised and deprotected to yield compound **27** [SEQ ID NO:60].

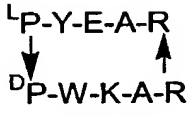
MW: $C_{54}H_{88}N_{18}O_{13}$, [1197.4]

MS(ESI): 599.4 $[M+2H^{+}]^{2+}$

HPLC-RT(min.): 8.85

Conditions: Analytical HPLC-conditions: column CC 250/4 Nucleosil 100-5 Protect 1 (Ser. No. 8111346, Batch 8023); gradient: 10 % acetonitril / 90 % water(containing 0.1% trifluoroacetic acid) to 100% acetonitril in 15 minutes; stay constant for 4 minutes and return to10 % acetonitril / water(0.1% trifluoroacetic acid) in 4 minutes.

Example 28:



28

By a procedure analogous to that described in Example 1, Fmoc-Ala-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-^{L}Pro-OH, Fmoc-^{D}Pro-OH, Fmoc-Trp(Boc)-OH, Fmoc-Lys(Boc)-OH,

Fmoc-Ala-OH and Fmoc-Arg(Pmc)-OH were coupled to the Fmoc-Arg(Pmc)-OH loaded resin, cleaved, cyclised and deprotected to yield compound **28** [SEQ ID NO:61].

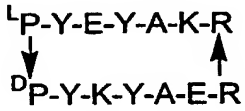
MW: $C_{59}H_{87}N_{18}O_{13}$, [1256.4]

MS(ESI): 628.50 $[M+2H^+]^{2+}$, 419.20 $[M+3H^+]^{3+}$

HPLC-RT(min.): 9.16

Conditions: Analytical HPLC-conditions: column CC 250/4 Nucleosil 100-5 Protect 1 (Ser. No. 8111346, Batch 8023); gradient: 10 % acetonitril / 90 % water(containing 0.1% trifluoroacetic acid) to 100% acetonitril in 15 minutes; stay constant for 4 minutes and return to10 % acetonitril / water(0.1% trifluoroacetic acid) in 4 minutes.

Example 29:



29

By a procedure analogous to that described in Example 1, Fmoc-Lys(Boc)-OH, Fmoc-Ala-OH, Fmoc-Tyr(OtBu)-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-^{L}Pro-OH, Fmoc-^{D}Pro-OH, , Fmoc-Tyr(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Ala-OH, Fmoc-Glu(OtBu)-OH and Fmoc-Arg(Pmc)-OH were coupled to the Fmoc-Arg(Pmc)-OH loaded resin, cleaved, cyclised and deprotected to yield compound **29** [SEQ ID NO:62].

MW: $C_{86}H_{122}N_{22}O_{22}$, [1816]

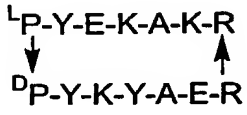
MS(ESI): 908 $[M+2H^+]^{2+}$, 606.2 $[M+3H^+]^{3+}$

HPLC-RT(min.): 8.40

Conditions: Analytical HPLC-conditions: column CC 250/4 Nucleosil 100-5 Protect 1 (Ser. No. 8111346, Batch 8023); gradient: 10 % acetonitril / 90 % water(containing 0.1% trifluoroacetic acid) to 100% acetonitril in 15 minutes; stay constant for

4 minutes and return to10 % acetonitril / water(0.1% trifluoroacetic acid) in 4 minutes.

Example 30:



30

By a procedure analogous to that described in Example 1, Fmoc-Lys(Boc)-OH, Fmoc-Ala-OH, Fmoc-Lys(Boc)-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-^{L}Pro-OH, Fmoc-^{D}Pro-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Ala-OH, Fmoc-Glu(OtBu)-OH and Fmoc-Arg(Pmc)-OH were coupled to the Fmoc-Arg(Pmc)-OH loaded resin, cleaved, cyclised and deprotected to yield compound **30** [SEQ ID NO:63].

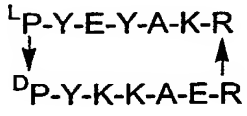
MW: $C_{83}H_{125}N_{23}O_{21}$, [1781]

MS(ESI): 594.6 $[M+3H^{+}]^{3+}$

HPLC-RT(min.): 9.04

Conditions: Analytical HPLC-conditions: column CC 250/4 Nucleosil 100-5 Protect 1 (Ser. No. 8111346, Batch 8023); gradient: 10 % acetonitril / 90 % water(containing 0.1% trifluoroacetic acid) to 100% acetonitril in 15 minutes; stay constant for 4 minutes and return to10 % acetonitril / water(0.1% trifluoroacetic acid) in 4 minutes.

Example 31:



31

By a procedure analogous to that described in Example 1, Fmoc-Lys(Boc)-OH, Fmoc-Ala-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-^{L}Pro-OH, Fmoc-^{D}Pro-

OH, Fmoc-Tyr(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ala-OH, Fmoc-Glu(OtBu)-OH and Fmoc-Arg(Pmc)-OH were coupled to the Fmoc-Arg(Pmc)-OH loaded resin, cleaved, cyclised and deprotected to yield compound **31** [SEQ ID NO:64]

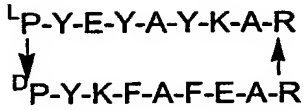
MW: $C_{83}H_{125}N_{23}O_{21}$, [1721]

MS(ESI): 891.15 $[M+2H^+]^{2+}$, 594.85 $[M+3H^+]^{3+}$

HPLC-RT(min.): 9.84

Conditions: Analytical HPLC-conditions: column CC 250/4 Nucleosil 100-5 Protect 1 (Ser. No. 8111346, Batch 8023); gradient: 10 % acetonitril / 90 % water(containing 0.1% trifluoroacetic acid) to 100% acetonitril in 15 minutes; stay constant for 4 minutes and return to10 % acetonitril / water(0.1% trifluoroacetic acid) in 4 minutes.

Example 32:



32

By a procedure analogous to that described in Example 1, Fmoc-Ala-OH, Fmoc-Lys(Boc)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Ala-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-^{L}Pro-OH, Fmoc-^{D}Pro-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Phe-OH, Fmoc-Ala-OH, Fmoc-Phe-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Ala-OH, and Fmoc-Arg(Pmc)-OH were coupled to the Fmoc-Arg(Pmc)-OH loaded resin, cleaved, cyclised and deprotected to yield compound **32** [SEQ ID NO:65]

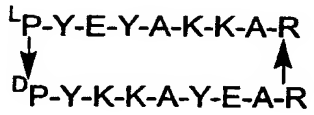
MW: $C_{110}H_{150}N_{26}O_{26}$, [2252.4]

MS(ESI): 751.93 $[M+3H^+]^{3+}$

HPLC-RT(min.): 9.42

Conditions: Analytical HPLC-conditions: column CC 250/4 Nucleosil 100-5 Protect 1 (Ser. No. 8111346, Batch 8023); gradient: 10 % acetonitril / 90 % water(containing 0.1% trifluoroacetic acid) to 100% acetonitril in 15 minutes; stay constant for 4 minutes and return to10 % acetonitril / water(0.1% trifluoroacetic acid) in 4 minutes.

Example 33:



33

By a procedure analogous to that described in Example 1, Fmoc-Ala-OH, Fmoc-Lys(Boc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ala-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-^{L}Pro-OH, Fmoc-^{D}Pro-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ala-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Ala-OH, and Fmoc-Arg(Pmc)-OH were coupled to the Fmoc-Arg(Pmc)-OH loaded resin, cleaved, cyclised and deprotected to yield compound **33** [SEQ ID NO:66].

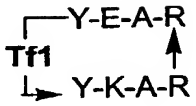
MW: $C_{104}H_{156}N_{28}O_{26}$, [2214.5]

MS(ESI): 738.10 $[M+3H^+]^{3+}$

HPLC-RT(min.): 13.46

Conditions: Analytical HPLC-conditions: column CC 250/4 Nucleosil 100-5 Protect 1 (Ser. No. 8111346, Batch 8023); gradient: 10 % acetonitril / 90 % water(containing 0.1% trifluoroacetic acid) to 100% acetonitril in 15 minutes; stay constant for 4 minutes and return to10 % acetonitril / water(0.1% trifluoroacetic acid) in 4 minutes.

Example 34:



34

By a procedure analogous to that described in Example 1, Fmoc-Ala-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Tyr(tBu)-OH, Template **f1**, Fmoc-Tyr(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ala-OH, and Fmoc-Arg(Pmc)-OH were coupled to the Fmoc-Arg(Pmc)-OH loaded resin, cleaved, cyclised and deprotected to yield compound **34** [SEQ ID NO:67].

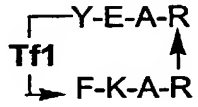
MW: $C_{67}H_{91}N_{16}O_{16}$, [1376.5]

MS(ESI): 689.02 $[M+2H^+]^{2+}$

HPLC-RT(min.): 9.87

Conditions: Analytical HPLC-conditions: column CC 250/4 Nucleosil 100-5 Protect 1 (Ser. No. 8111346, Batch 8023); gradient: 10 % acetonitril / 90 % water(containing 0.1% trifluoroacetic acid) to 100% acetonitril in 15 minutes; stay constant for 4 minutes and return to10 % acetonitril / water (0.1% trifluoroacetic acid) in 4 minutes.

Example 35:



35

By a procedure analogous to that described in Example 1, Fmoc-Ala-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Tyr(tBu)-OH, Template **f1**, Fmoc-Phe-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ala-OH, and Fmoc-Arg(Pmc)-OH were coupled to the Fmoc-Arg(Pmc)-OH loaded resin, cleaved, cyclised and deprotected to yield compound **35** [SEQ ID NO:68].

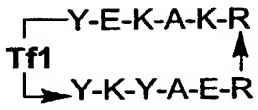
MW: $C_{67}H_{91}N_{16}O_{15}$, [1360.14]

MS(ESI): 681.44 $[M+2H^+]^{2+}$, 454.77 $[M+3H^+]^{3+}$

HPLC-RT(min.): 9.68

Conditions: Analytical HPLC-conditions: column CC 250/4 Nucleosil 100-5 Protect 1 (Ser. No. 8111346, Batch 8023); gradient: 10 % acetonitril / 90 % water(containing 0.1% trifluoroacetic acid) to 100% acetonitril in 15 minutes; stay constant for 4 minutes and return to10 % acetonitril / water(0.1% trifluoroacetic acid) in 4 minutes.

Example 36:



36

By a procedure analogous to that described in Example 1, Fmoc-Lys(Boc)-OH, Fmoc-Ala-OH, Fmoc-Lys(Boc)-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Tyr(tBu)-OH, Template **f1**, Fmoc-Tyr(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Ala-OH, Fmoc-Glu(OtBu)-OH, and Fmoc-Arg(Pmc)-OH were coupled to the Fmoc-Arg(Pmc)-OH loaded resin, cleaved, cyclised and deprotected to yield compound **36** [SEQ ID NO:69].

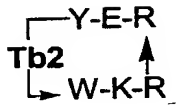
MW: $C_{93}H_{131}N_{22}O_{23}$, [1925.19]

MS(ESI): 643.28 $[M+3H^+]^{3+}$

HPLC-RT(min.): 8.85 min.

Conditions: Analytical HPLC-conditions: column CC 250/4 Nucleosil 100-5 Protect 1 (Ser. No. 8111346, Batch 8023); gradient: 10 % acetonitril / 90 % water(containing 0.1% trifluoroacetic acid) to 100% acetonitril in 15 minutes; stay constant for 4 minutes and return to10 % acetonitril / water(0.1% trifluoroacetic acid) in 4 minutes.

Example 37:



37

By a procedure analogous to that described in Example 1, Fmoc-Glu(OtBu)-OH, Fmoc-Tyr(tBu)-OH, Template **b1**, Fmoc-Trp(Boc)-OH, Fmoc-Lys(Boc)-OH and Fmoc-Arg(Pmc)-OH were coupled to the Fmoc-Arg(Pmc)-OH loaded resin, cleaved, cyclised and deprotected to yield compound **37** [SEQ ID NO:70].

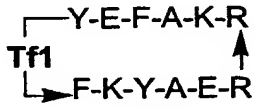
MW: $C_{54}H_{74}N_{17}O_{14}$, [1185.18]

MS(ESI): 593.83 $[M+2H^+]^{2+}$

HPLC-RT(min.): 11.23

Conditions: Analytical HPLC-conditions: column CC 250/4 Nucleosil 100-5 Protect 1 (Ser. No. 8111346, Batch 8023); gradient: 10 % acetonitril / 90 % water(containing 0.1% trifluoroacetic acid) to 100% acetonitril in 15 minutes; stay constant for 4 minutes and return to10 % acetonitril / water(0.1% trifluoroacetic acid) in 4 minutes.

Example 38



38

By a procedure analogous to that described in Example 1, Fmoc-Lys(Boc)-OH, Fmoc-Ala-OH, Fmoc-Phe-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Tyr(tBu)-OH, Template **f1**, Fmoc-Phe-OH, Fmoc-Lys(Boc)-OH, Fmoc-Tyr(OtBu)-OH, Fmoc-Ala-OH, Fmoc-Glu(OtBu)-OH and Fmoc-Arg(Pmc)-OH were coupled to the Fmoc-Arg(Pmc)-OH loaded resin, cleaved, cyclised and deprotected to yield compound **38** [SEQ ID NO:71].

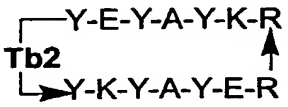
MW: $C_{96}H_{129}N_{21}O_{22}$, [1929.23]

MS(ESI): 644 $[M+3H^+]^{3+}$, 483.11 $[M+4H^+]^{4+}$

HPLC-RT(min.): 9.22

Conditions: Analytical HPLC-conditions: column CC 250/4 Nucleosil 100-5 Protect 1 (Ser. No. 8111346, Batch 8023); gradient: 10 % acetonitril / 90 % water(containing 0.1% trifluoroacetic acid) to 100% acetonitril in 15 minutes; stay constant for 4 minutes and return to10 % acetonitril / water(0.1% trifluoroacetic acid) in 4 minutes.

Example 39:



39

By a procedure analogous to that described in Example 1, Fmoc-Lys(Boc)-OH, Fmoc-Tyr(tBu)-OH Fmoc-Ala-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Tyr(tBu)-OH, Template **b1**, Fmoc-Tyr(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Tyr(OtBu)-OH, Fmoc-Ala-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Glu(OtBu)-OH and Fmoc-Arg(Pmc)-OH were coupled to the Fmoc-Arg(Pmc)-OH loaded resin, cleaved, cyclised and deprotected to yield compound **39** [SEQ ID NO:72].

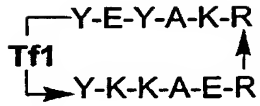
MW: $C_{105}H_{138}N_{25}O_{29}$, [2214.3]

MS(ESI): 737.76 $[M+3H^+]^{3+}$

HPLC-RT(min.): 13.26

Conditions: Analytical HPLC-conditions: column CC 250/4 Nucleosil 100-5 Protect 1 (Ser. No. 8111346, Batch 8023); gradient: 10 % acetonitril / 90 % water(containing 0.1% trifluoroacetic acid) to 100% acetonitril in 15 minutes; stay constant for 4 minutes and return to10 % acetonitril / water(0.1% trifluoroacetic acid) in 4 minutes.

Example 40



40

By a procedure analogous to that described in Example 1, Fmoc-Lys(Boc)-OH, Fmoc-Ala-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Tyr(tBu)-OH, Template **f1**, Fmoc-Tyr(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ala-OH, Fmoc-Glu(OtBu)-OH and Fmoc-Arg(Pmc)-OH were coupled to the Fmoc-Arg(Pmc)-OH loaded resin, cleaved, cyclised and deprotected to yield compound **40** [SEQ ID NO:73].

MW: $C_{93}H_{131}N_{22}O_{23}$, [1926.22]

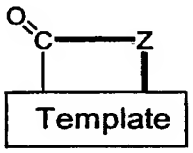
MS(ESI): 643.01 $[M+3H^+]^{3+}$, 482.35 $[M+4H^+]^{4+}$

HPLC-RT(min.): 8.99

Conditions: Analytical HPLC-conditions: column CC 250/4 Nucleosil 100-5 Protect 1 (Ser. No. 8111346, Batch 8023); gradient: 10 % acetonitril / 90 % water(containing 0.1% trifluoroacetic acid) to 100% acetonitril in 15 minutes; stay constant for 4 minutes and return to10 % acetonitril / water(0.1% trifluoroacetic acid) in 4 minutes.

CLAIMS

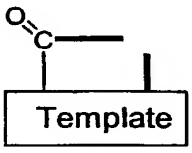
1. A process for the manufacture of compounds of the general formula



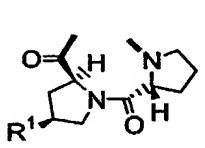
I

wherein

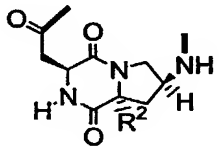
Z is a chain of n α-amino acid residues which, if their α-C atom is asymmetric, have L-configuration, n being an integer from 4 to 20, the positions of said amino acid residues in said chain being counted starting from the N-terminal amino acid;



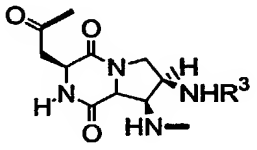
is one of the groups of formulae



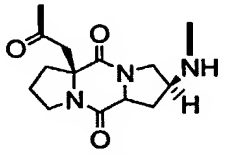
(a)



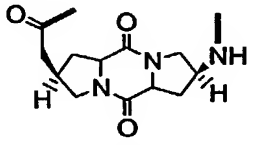
(b)



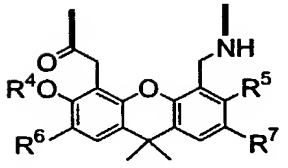
(c)



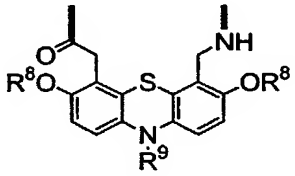
(d)



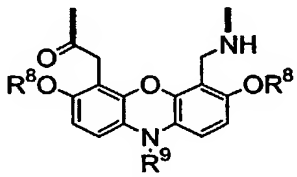
(e)



(f)



(g)



(h)

R^1 is hydrogen or a protected amino group;

R^2 is hydrogen or a group of formula CH_2-$COOR^{10}$;

R^3 is an amino-protecting group;

R^4 is lower alkyl or aryl-lower alkyl;

R^5 is lower alkyl, lower alkoxy or aryl;

R^6 is hydrogen, lower alkyl, substituted lower alkyl, aryl, Br or NO_2;

R^7 is hydrogen, lower alkyl, substituted lower alkyl, aryl, Br or NO_2;

R^8 is lower alkyl, substituted lower alkyl or aryl-lower alkyl;

R^9 is lower alkyl, substituted lower alkyl or aryl-lower alkyl; and

R^{10} is hydrogen, lower alkyl, substituted lower alkyl, aryl, aryl-lower alkyl, aroyl-lower alkyl or allyl;

and of salts thereof, which process comprises

(a) coupling an appropriately functionalized solid support with an appropriately N-protected derivative of that amino acid which in the desired end-product is in position $^n/_2$, $^n/_2+1$ or $^n/_2-1$ if n is an even number and, respectively, in position $^n/_2\pm^1/_2$ or $^n/_2-^1/_2$ if n is an odd number, any functional group which may be present in said N-protected amino acid derivative being likewise appropriately protected;

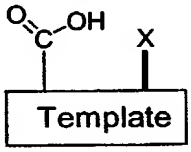
(b) removing the N-protecting group from the product thus obtained;

(c) coupling the product thus obtained with an appropriately N-protected derivative of that amino acid which in the desired end-product is one position nearer the N-terminal amino acid residue, any functional group which may be present in said N-protected amino acid derivative being likewise appropriately protected;

(d) removing the N-protecting group from the product thus obtained;

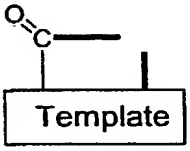
(e) repeating, if necessary, steps (c) and (d) until the N-terminal amino acid residue has been introduced;

(f) coupling the product thus obtained with a compound of the general formula

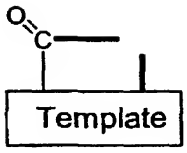


II

wherein

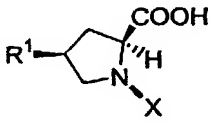


is as defined above and X is an N-protecting group or, if



is to be group (a), above, alternatively

(fa) coupling the product obtained in step (d) or (e) with a compound of the general formula **III**



III

wherein R^1 and X are as defined above;

(fb) removing the N-protecting group from the product thus obtained; and

(fc) coupling the product thus obtained with an appropriately N-protected derivative of D-proline;

(g) removing the N-protecting group from the product obtained in step (f) or (fc);

(h) coupling the product thus obtained with an appropriately N-protected derivative of that amino acid which in the desired end-product is in position n, any functional group which may be present in said N-protected amino acid derivative being likewise appropriately protected;

(i) removing the N-protecting group from the product thus obtained;

(j) coupling the product thus obtained with an appropriately N-protected derivative of that amino acid which in the desired end-product is one position farther away from position n, any functional group which may be present in said N-protected amino acid derivative being likewise appropriately protected;

(k) removing the N-protecting group from the product thus obtained;

(l) repeating, if necessary, steps (j) and (k) until all amino acid residues have been introduced;

(m) detaching the product thus obtained from the solid support;

(n) cyclising the product cleaved from the solid support;

(o) removing any protecting groups present on functional groups of any members of the chain of amino acid residues and, if desired, any protecting group(s) which may in addition be present in the molecule; and

(p) if desired, converting the product thus obtained into a salt or converting a salt thus obtained into the corresponding free compound of formula I or into a different salt.

2. A process according to claim 1 wherein the functionalized solid support is derived from polystyrene crosslinked with divinylbenzene; from polystyrene coated with polyethyleneglycol spacers; or from a polyacrylamide resin; and is functionalized by means of a linker, i. e. a bifunctional spacer molecule which contains on one end an anchoring group for attachment to the solid support and on the other end a selectively cleavable functional group used for the subsequent chemical transformation and cleavage procedures.

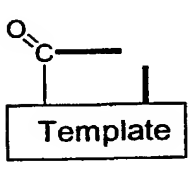
3. A process according to claim 2 wherein the linker forms acid-labile benzyl, benzhydryl or trityl esters with the carboxyl group of the amino acids.

4. A process according to claim 3 wherein a 3-methoxy-4-hydroxyphenylphenoxy (Sasrin), 4-(2,4-dimethoxyphenyl-hydroxymethyl)-phenoxy (Rink), 4-(4-hydroxymethyl-3-methoxy-phenoxy)butyric acid (HMPB), trityl or 2-chlorotrityl linker is used.

5. A process according to any one of claims 1 to 4 wherein X and the N-protecting group of the amino acid derivatives is 9-fluorenylmethoxycarbonyl (Fmoc).

6. A modification of the process according to any one of claims 1 to 5 for the manufacture of enantiomers of the compounds of formula I as defined in claim 1 in which all amino acids which have an asymmetric α-carbon atom are used in their D-Form and the enantiomer of a template corresponding to structure (a), (b), (c), (d) or (e) or a template corresponding to formula (f), (g) or (h) is used in step (f) and, respectively, the enantiomer of a compound of formula III is used in step (fa) and a derivative of L-proline is used in step (fc).

7. Compounds of the general formula I as defined in Claim 1 with the provisos that if



is

(i) group (a) and R^1 is hydrogen, then Z is other than

-Val-Lys-Asn-Tyr-Gly-Val-Lys-Asn-Ser-Glu-Trp-Ile- [SEQ ID NO:9],
-Val-Lys-Asn-Tyr-Gly-Val-Lys-Asn-Ser-Glu-Trp-Thr- [SEQ ID NO:10],
-Gly-Arg-Gly-Asp- [SEQ ID NO:11],
-Arg-Gly-Asp-Gly- [SEQ ID NO:12],
-Phe-Tyr-Thr-Gly-Thr- [SEQ ID NO:13],
-Tyr-Arg-Asp-Ala-Met- [SEQ ID NO:14],
-Asn-Thr-Tyr-Ser-Gly-Val- [SEQ ID NO:15],
-Trp-Asp-Asp-Gly-Ser-Asp- [SEQ ID NO:16] and
-Leu-Trp-Tyr-Ser-Asn-His-Trp-Val- [SEQ ID NO:17];

(ii) group (b) and R^2 is hydrogen or CH_2-COOH, or group (c) and R^3 is benzoyl, or group (d), or group (e), then Z is other than -Ala-Asn-Pro-Asn-Ala-Ala- [SEQ ID NO:18];

(iii) group (b) and R^2 is hydrogen, then Z is other than -Ala-Arg-Gly-Asp- [SEQ ID NO:19];

(iv) group (f), R^4 is methyl, R^5 is methoxy and R^6 and R^7 each are hydrogen, then Z is other than

-Val-Ala-Ala-Phe-Leu-Ala-Leu-Ala- [SEQ ID NO:20],
-Arg-Gly-Asp-Val- [SEQ ID NO:21],
-Ala-Thr-Val-Gly- [SEQ ID NO:22],
-Glu-Arg-Gly-Asp-Val-Tyr- [SEQ ID NO:23],
-Ile-Ala-Arg-Gly-Asp-Phe-Pro-Asp- [SEQ ID NO:24],
-Ala-Arg-Ile-Ala-Arg-Gly-Asp-Phe-Pro-Asp-Asp-Arg- [SEQ ID NO:25],
-Ala-Arg-Gly-Asp-Phe-Pro- [SEQ ID NO:26],
-Arg-Gly-Asp-Phe- [SEQ ID NO:27] and
-Arg-Ile-Ala-Arg-Gly-Asp-Phe-Pro-Asp-Asp- [SEQ ID NO:28];

(v) group (g), R^8 is methyl and R^9 is methyl or n-hexyl, or group (h), R^8 is ethyl and R^9 is ethyl, then Z is other than -Arg-Gly-Asp-Val- [SEQ ID NO:21];

(vi) group (g), R^8 is methyl and R^9 is methyl or benzyl, then Z is other than -Gly-Gly-Ala-Gly- [SEQ ID NO:29];

(vii) group (g), R^8 is methyl and R^9 is methyl, then Z is other than -Gly-Asp-Gly-Gly- [SEQ ID NO:30]; and

(viii) group (g), R^8 is methyl and R^9 is n-hexyl, then Z is other than -Val-Arg-Lys-Lys- [SEQ ID NO:1].

8. The enantiomers of the compounds of the general formula I as defined in claim 1.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
8 March 2001 (08.03.2001)

PCT

(10) International Publication Number
WO 01/16161 A1

(51) International Patent Classification[7]: C07K 7/02, 7/06, 7/08, 7/64, 1/04

(21) International Application Number: PCT/EP99/06369

(22) International Filing Date: 30 August 1999 (30.08.1999)

(25) Filing Language: English

(26) Publication Language: English

(71) Applicant *(for all designated States except US)*: **POLYPHOR AG** [CH/CH]; Wintherthurerstrasse 190, CH-8057 Zürich (CH).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **ROBINSON, John, A.** [GB/CH]; Institute of Organic Chemistry, University of Zürich, Winterthurerstrasse 190, CH-8057 Zürich (CH). **OBRECHT, Daniel** [CH/CH]; Seltisbergerstrasse 30, CH-4059 Basel (CH).

(74) Agent: **BRAUN, André**; Braun & Partner, Reussstrasse 22, CH-4054 Basel (CH).

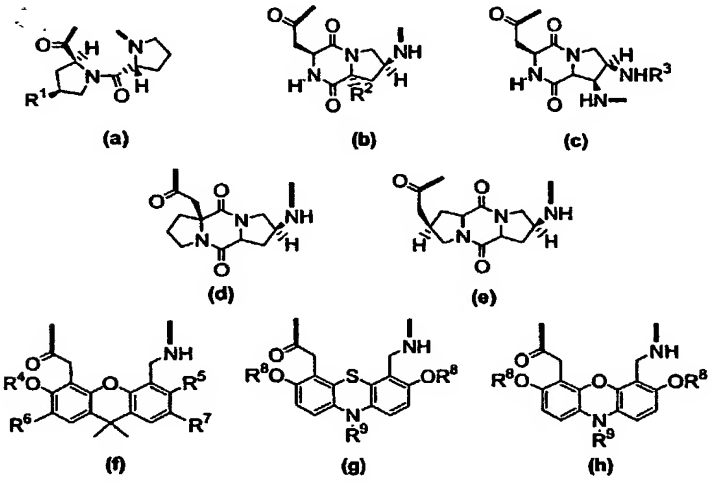
(81) Designated States *(national)*: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

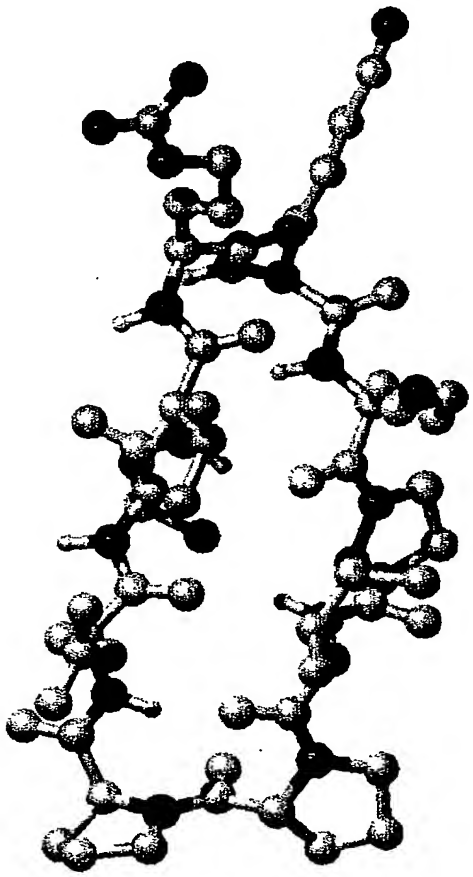
(54) Title: SYNTHESIS OF TEMPLATE-FIXED β-HAIRPIN LOOP MIMETICS



(57) Abstract: Template-fixed β-hairpin loop mimetics comprising a template corresponding to one of the structures (a), (b), (c), (d), (e), (f), (g), (h) and a template-fixed chain of 4 to 20 α-amino acid residues which, if their α-C atom is asymmetric, have L-configuration can be manufactured by a novel process which is based on a mixed solid- and solution phase synthetic strategy. If desired, this process can be modified to give the enantiomers of these template-fixed β-hairpin loop mimetics. These enantiomers are novel compounds, and many of said template-fixed β-hairpin loop mimetics themselves are also novel compounds. The template-fixed β-hairpin loop mimetics and their enantiomers can mimick flat surfaces of proteins and thus be used to probe large surface protein-protein interactions. Accordingly they can serve as lead finding tools for protein targets where it is difficult to find small-molecular-weight lead compounds.

WO 01/16161 A1

Figure. Solution conformation of Example-1. The D-Pro-L-Pro template is at the bottom. N-atoms are in black, other atoms in grey.



PATENT

Attorney's Docket No.

COMBINED DECLARATION AND POWER OF ATTORNEY

(ORIGINAL, DESIGN, NATIONAL STAGE OF PCT, SUPPLEMENTAL, DIVISIONAL, CONTINUATION OR CIP)

As a below named inventor, I hereby declare that:

TYPE OF DECLARATION

This declaration is of the following type: *(check one)*

- ☐ Original
- ☐ Supplemental
- ☐ Design
- ☒ National Stage PCT
- ☐ Divisional
- ☐ Continuation
- ☐ Continuation-in-Part (CIP)

INVENTORSHIP IDENTIFICATION

NOTE: If the inventors are each not the inventors of all the claims an explanation of the facts, including the ownership of all the claims at the time the last claimed invention was made, should be submitted.

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

SYNTHESIS OF TEMPLATE-FIXED β-HAIRPIN LOOP MIMETICS

the specification of which: *(complete (a), (b) or (c))*

(a) ☐ is attached hereto.

(b) ☐ was filed on _____ as
☐ Serial No. _____ or
☐ Express Mail No. _____, as Serial No. not yet known
and was amended on _____. *(if applicable)*

(c) ☒ was described and claimed in PCT International Application No. PCT/EP99/06369
filed on 30/8/1999 and as amended under PCT Article 19 on _____. *(if any)*

ACKNOWLEDGMENT OF REVIEW OF PAPERS AND DUTY OF CANDOR

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above, and that the filing of said specification, if heretofore filed, was authorized by me.

I acknowledge the duty to disclose information which is material to the patentability of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

CLAIM OF PRIORITY OF EARLIER FOREIGN APPLICATION(S) UNDER 35 U.S.C. §119(a)-(d)

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

(List prior foreign/PCT application(s) filed within 12 months (6 months for design) prior to this U.S. application.)

NOTE: Where item (c) is entered above and the International Application which designated the U.S. claimed priority check item (e), enter the details below and make the priority claim.

COUNTRY (orPCT)	APPLICATION NO.	DATE OF FILING *(Day/Month/Year)*	PRIORITY CLAIMED UNDER 35 USC §119
			☐ YES ☐ NO
			☐ YES ☐ NO

CLAIM FOR BENEFIT OF PRIOR U.S. PROVISIONAL APPLICATION(S) UNDER 35 U.S.C. §119(e)

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below:

(List prior U.S. provisional applications.)

PROVISIONAL APPLICATION NO.	FILING DATE *(Day/Month/Year)*

CLAIM FOR BENEFIT OF EARLIER U.S./PCT APPLICATION(S) UNDER 35 U.S.C. 120

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in such prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:

(List prior U.S. applications or PCT international applications designating the U.S. for benefit under 35 U.S.C. §120.)

U.S. APPLICATIONS		STATUS *(Check One)*		
U.S. SERIAL NO.	U.S. FILING DATE *(Day/Month/Year)*	Patented	Pending	Abandoned
0/		☐	☐	☐
0/		☐	☐	☐

PCT APPLICATIONS DESIGNATING THE U.S.			STATUS *(Check One)*		
PCT APPLN. NO.	PCT FILING DATE *(Day/Month/Year)*	U.S. SERIAL NOS ASSIGNED (If any)	Patented	Pending	Abandoned
PCT/			☐	☐	☐
PCT/			☐	☐	☐

35 USC 119 PRIORITY CLAIM, IF ANY, FOR ABOVE LISTED U.S./PCT APPLICATIONS

PRIORITY APPLICATION NO.	PRIORITY COUNTRY	FILING DATE *(Day/Month/Year)*	ISSUE DATE *(Day/Month/Year)*

POWER OF ATTORNEY

As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office in connection therewith:

(32) Charles R. Hoffmann, Reg. No. 24,102; Ronald J. Baron, Reg. No. 29,281; Gerald T. Bodner, Reg. No. 30,449; Alan M. Sack, Reg. No. 31,874; A. Thomas Kammer, Reg. No. 28,226; R. Glenn Schroeder, Reg. No. 34,720; Glenn T. Henneberger, Reg. No. 36,074; Irving N. Feit, Reg. No. 28,601; Anthony E. Bennett, Reg. No. 40,910; Gregory W. Bachmann, Reg. No. 41,593; Steven T. Zuschlag, Reg. No. 43,309; Susan A. Sipos, Reg. No. 43,128, Kevin E. McDermott, Reg. No. 35,946; Robert C. Morriss, Reg. No. 42,910; Roderick S.W. Turner, Reg. No. 38,639; James F. Harrington, Reg. No. 44,741; Samir R. Patel, Reg. No. 44,998, Richard LaCava, Reg. No. 41,135; Algis Anilionis, Reg. No. 36,995; Justin K. Holmes, Reg. No. 42,666; Joseph J. Catanzaro, Reg. No. 25,837; and Robert L. Bernstein, Reg. No. P-46,020, each of them of HOFFMANN & BARON, LLP, 6900 Jericho Turnpike, Syosset, New York 11791; and Daniel A. Scola, Jr., Reg. No. 29,855; Salvatore J. Abbruzzese, Reg. No. 30,152; Kirk M. Miles, Reg. No. 37,891; Robert F. Chisholm, Reg. No. 39,939; Kellyanne Merkel, Reg. No. 43,800; Keith R. Lange, Reg. No. 44,201; John Sopko, Reg. No. 41,321; Barry Jacobsen, Reg. No. 43,689; Gloria K. Szakiel, Reg. No. 45,149; and Mark E. Baron, Reg. No. 46,150, each of them of HOFFMANN & BARON, LLP, 1055 Parsippany Boulevard, Parsippany, New Jersey 07054.

PLEASE SEND CORRESPONDENCE TO:	PLEASE DIRECT TELEPHONE CALLS TO:
Daniel A. Scola, Jr. HOFFMANN & BARON, LLP 6900 Jericho Turnpike Syosset, New York 11791	Daniel A. Scola, Jr. (973) 331-1700

DECLARATION

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

SIGNATURE(S)

1-00

Full Name of Sole or First Inventor: John A. Robinson

Country of Citizenship: Great Britain

Residence Address: Institut of Organic Chemistry,,University of Zurich, Winterthurerstrasse 190, CH-8057 Zürich CHX

Post Office Address:

Date: 4.01.02 Inventor's signature

2-00

Full Name of Sole or First Inventor: Daniel Obrecht

Country of Citizenship: Switzerland

Residence Address: Seltisbergerstrasse 30
CH-4059 Basel / Switzerland CHX

Post Office Address:

Date: 4.1.02 Inventor's signature

NOTE: All above spaces identifying inventors must be completed or deleted before any inventor executes this application